THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today (1) was not written for publication in a law journal or reporter and (2) is not binding precedent of the Board.

Filed by: Merits Panel
Box Interference
Washington, D.C. 20231
Tel: (703)308-9797
Fax: (703)308-7953

Paper 365

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

EVA S. ENGVALL, ERIKKI I. RUOSLAHTI and MARJATTA UOTILA

Junior Party,[1]

v.

GARY S. DAVID and HOWARD E. GREENE

Senior Party.[2]

Patent Interference 101,769

Before: DOWNEY, Administrative Patent Judge, McKELVEY, Senior Administrative Patent Judge, and SCHAFER, Administrative Patent Judge.

SCHAFER, Administrative Patent Judge

FINAL JUDGMENT AND OPINION

---

[1] Application 06/539,754, filed October 6, 1983.

[2] Patent 4,376,110 issued March 8, 1983, based on application 06/175,133 filed August 4, 1980.

TABLE OF CONTENTS

Page No.

DECISION AND JUDGMENT

This interference is between an application filed by Engvall et al. (Engvall) and a patent to David et al. (David). The real parties in interest are the respective assignees, La Jolla Cancer Research Foundation (La Jolla) and Hybritech, Inc. (Hybritech). We award judgment against Engvall. Therefore, Engvall is not entitled to a patent claiming the subject matter set out in claims 1 to 45 of application 06/539,754. David is entitled to claims 1 to 29 of patent 4,376,110.

BACKGROUND

The David patent issued on March 8, 1983. The patent has resulted in at least two appeals to the United States Court of Appeal for the Federal Circuit, Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987) and Hybritech Inc. v. Abbott Laboratories, 849 F.2d 1446, 7 USPQ2d 1191 (Fed. Cir. 1988).

ISSUES

The following issues were raised by the parties at final hearing:

1. Are Engvall's claims 8 to 27 unpatentable under 35 U.S.C. § 112, ¶1, for failure to satisfy the written description requirement?

2. Has Engvall proved priority of invention with respect to the subject matter of the count?

3. Has Engvall proved inequitable conduct by David?

THE SUBJECT MATTER OF THE INTERFERENCE

The sole count in this interference provides:

> In an immunometric assay to determine the presence or concentration of an antigenic substance in a sample of a fluid comprising forming a ternary complex of
>
> a first labeled antibody,
> said antigenic substance, and
> a second antibody
>
> said second antibody being bound to a solid carrier insoluble in said fluid
>
> wherein the presence of the antigenic substance in the samples is determined by measuring either the amount of labeled antibody bound to the solid carrier or the amount of unreacted labeled antibody,

> the improvement comprising employing monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole for each of said labeled antibody and said antibody bound to a solid carrier. [Emphasis added.]

We interpret the limitation "10^8 liters/mole" to mean $1x10^8$ liters/mole.[3] The phrase "at least" indicates that the stated value for the affinity constant is a minimum value. The effect of the word "about" is to broaden the count to encompass values of the affinity constant somewhat lower than $1x10^8$ liters/mole.

Prior to the invention of the subject matter of the count, immunometric or sandwich assays used polyclonal antibodies. The improvement recited in the count resides partially in the substitution of monoclonal antibodies for polyclonals. It also resides in the use of ?monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole for each of said labeled antibody and said antibody bound to a solid carrier." Thus, the count does not encompass the use of all monoclonal antibodies and their respective antigens. It is limited to the subgenus of monoclonals and antigens having an affinity constant beginning at about 1 x 10^8 liters/mole and ranging up to infinity. In other words "about 10^8 liters per mole" sets the minimum of the affinity range.

Engvall's claims 1 to 45 and David's claims 1 to 29 correspond to the sole count of this interference.

---

[3] To interpret this limitation any other way would render the limitation meaningless. In scientific notation 5 X 10^8 may also be properly written as 50 X 10^7 or 0.5 X 10^9.

OPINION[4]

I. Technical background [5]

The subject matter of the count is directed to an improvement in immunometric or sandwich assays. Such assays are used to detect or quantitate the presence of specific antigens in a fluid. Antigens are molecular configurations that the immune system recognizes as foreign substances and which elicit an immune response.[6] Antigens are found on the surface of viruses, bacteria and other pathogens which invade mammalian bodies.[7] The immune response to foreign substances includes the production of antibodies. Antibodies, also called immunoglobulins (Ig), are proteins secreted into the bloodstream which seek out and mark antigens for destruction. [8] All antibodies have the same basic structure.[9] The basic structure is shown in the picture below:[10]

---

[4] Both Engvall and David have filed motions to suppress evidence. We address the specific decisions as applied to the evidence upon which we have relied as part of the discussion of or reference to that specific evidence. As to the matters raised at pages 17 to 22 of the David et al. Motion to Suppress Evidence (Paper 331) we consider the objections to have been waived as noted on page 18 of that paper.

[5] In the discussion of the technology we refer to the following reference works of which we take official notice of the scientific facts expressed therein (FRE 201):

Moore, Walter J., Physical Chemistry, 3d Ed., pp. 168-202, Prentice-Hall, Englewood Cliffs, New Jersey, 1964 (PHYCHEM)

Lewis, John R., First-Year College Chemistry, 7th Ed., pp.136-37, Barnes & Noble, New York, 1964 (CHEM);

Paul, William E., Fundamental Immunology, 3d Ed., pp. 422-433, Raven Press, New York, 1993 (FUND);

Roitt, Ivan et al., Immunology, 3d Ed., pp. 1.6-1.7 and 6.1-6.7, Mosby, London, 1993 (IMMU);

Watson, James et al., Recombinant DNA, 2d Ed. 1982, Scientific American Books, distributed by W.H.Freeman & Co., New York (DNA);

Darnell, James et al., Molecular Cell Biology, 2d Ed. 1990, Scientific American Books, distributed by W.H.Freeman & Co., New York (CELL)

A copy of the cited portions of these references is attached in the appendix to this opinion These references are not relied upon as prior art, but rather to assist the reader in the understanding the technology and terminology involved.

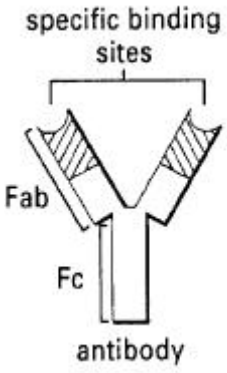
[6] DNA, pp. 293-94.

[7] IMMU, p. 1.7.

[8] DNA, p. 293.

[9] IMMU, p. 1.7

[10] IMMU, p. 1.6-1.7.



Antibodies have two Fab portions and a single Fc portion. The end of each Fab portion includes a binding site which binds to the antigen. See the figure above. The Fc portion interacts with other elements of the immune system.[11] In general, antibodies bind strongly with a particular portion of the antigen, called the antigenic determinant or epitope of the antigen.[12] An antigen may have several different epitopes or repeated epitopes.[13] Epitopes have a particular shape recognized by the binding site of the antibody.[14] Antibodies are specific to the particular epitopes with which they bind, rather than the antigen.[15]

When a pathogen is introduced into a body, the body's immune response produces a mixture of different **?**heterogenous" antibodies binding with different epitopes of the antigen.[16] In 1975, Köhler and Milstein, developed an *in vitro* technique for providing large quantities of homogeneous antibodies.[17] The antibodies produced by the Köhler and Milstein technique are now referred to as monoclonal antibodies.[18] The heterogenous mixtures of antibodies came to be known as polyclonal antibodies.

---

[11] IMMU, p. 1.6.

[12] IMMU, p. 1.7.

[13] IMMU, p. 1.7.

[14] IMMU, p. 1.8.

[15] IMMU, p. 1.7.

[16] FUND, pp. 421-22.

[17] Köhler, G. et al., **?**Continuous cultures of fused cells secreting antibody of predefined specificity," 256 Nature 495-97 (1975). .

[18] FUND, p. 455.

The binding strength between a single antibody and a single antigenic determinant is measured by the affinity constant for the antibody/antigen interaction.[19] The affinity constant is the association or equilibrium constant for the antibody/antigen reaction.[20] The magnitude of the affinity constant indicates the reactivity of an antibody with the antigen and the stability of the complex formed. Because the concept of the affinity constant is important to our decision we describe the concept in some detail.

The affinity constant is the equilibrium constant for the reaction of the antibody with the antigen of interest.[21] Such reactions are reversible.[22] Reversible reactions do not stop but reach a dynamic equilibrium in which the reaction rate in the forward and reverse directions are equal.[23] The reversible reaction of an antibody with the specific antigen may be represented by the following equation:[24]

$$Ab + Ag \rightleftharpoons Ab(Ag \quad .$$

Ab represents the antibody, Ag represents the antigen and Ab*Ag represents the antibody-antigen reaction product or complex.[25] The affinity constant for the reaction is the equilibrium constant, K, defined by the following equation:[26]

$$K = \frac{[Ab(Ag]}{[Ab] \cdot [Ag]}$$

.

---

19 IMMU, p. 6.3; 6.6; glossary.

20 FUND, p. 422.

21 IMMU, P. 6.3.

22 IMMU, p. 6.3; CELL, p. 1007; FUND, p. 422.

23 PHYCHEM, pp. 168-169.

24 IMMU, p. 6.3.

25 IMMU, p. 6.3.

26 IMMU, p. 6.3.

The bracketed terms, [Ab*Ag], [Ab] and [Ag], represent the equilibrium concentrations in moles[27] per liter of the antibody/antigen complex, the antibody and the antigen, respectively.[28] The "•" indicates the mathematical product of the adjacent terms. The units of affinity are liters/mole.[29] The calculated value of the affinity constant is a characteristic of the specific antibody/antigen reaction, rather than a characteristic of a particular antibody.[30]

As can be seen from the above equation, the mathematical maximum and minimum possibilities for affinity are ∞ and 0. Where all antibody and antigen bind to form Ab*Ag and remain bound (i.e., the binding is so strong that there is no reverse reaction), the concentration of antibody would be zero giving an affinity constant of ∞.[31] If no reaction takes place, there would be no concentration of product Ab*Ag, giving an affinity value of 0.[32, 33]

In reality, affinity falls between these two values. All antibody/antigen reactions have an affinity constant. The higher the affinity constant value, the tighter the binding of the antibody and the antigen and the lower the concentration of the antigen that can be detected.[34] The subject matter of this interference relates only to antibodies having an affinity constant of about 10^8 liters/mole and higher.

Avidity is a concept related to affinity. Because antibodies have two binding sites, antibodies are potentially multivalent in the reaction with antigen. Antigens may also have more than one antigenic determinant and may also be multivalent. When such a multivalent antigen combines with

---

[27] A mole is the weight in grams of 6.02×10^{23} molecules of a chemical. CHEM, p. 45.

[28] IMMU, p. 6.6

[29] $$\frac{[\text{moles/liter}]}{[\text{moles/liter}][\text{moles/liter}]} = \frac{1}{[\text{moles/liter}]} = \text{liters/mole}$$

[30] Throughout this opinion, the use of the word "affinity" means the affinity constant of the relevant antibody/antigen reaction.

[31] $$K = \frac{[Ab*Ag]}{[0]\cdot[0]} = \infty$$

[32] $$K = \frac{[0]}{[Ab][Ag]} = 0$$

[33] It is to be recognized that "0" and "∞" are theoretical mathematical limits and actually represent "non-reversible reactions."

[34] IMMU, p. 6.6.

more than one of an antibody's binding sites, the binding strength is referred to as avidity. Where multivalent bonding is involved the avidity is orders of magnitude greater than the sum of the affinities for each antigenic determinant and each antibody binding site.[35] Avidity is also relevant to antibodies bound to a substrate, such as used in immunometric assays. The close proximity of the immobilized antibodies to each other on the carrier surface tends to give an avidity or functional affinity which is substantially higher than the affinity constant for the free antibody.[36]

Specificity is another important property related to antibodies. Specificity is the ability of an antibody to discriminate between the antigen against which it was made (the immunogen) and any other antigen.[37] The converse of specificity is cross-reactivity, the tendency of an antibody to interact with antigens other than its immunogen.[38] Generally, the affinity constant for a cross-reaction is lower than the affinity constant for the reaction between an antibody and its immunogen.[39]

Immunoassays are diagnostic techniques which utilize the specificity and reactivity of antigens and antibodies to detect or quantitate antigens or antibodies in a solution.[40] Immunometric or "sandwich" assays of the type set forth in the count are used to determine the presence and/or concentration of a particular antigen in a fluid.[41] One type of immunometric assay uses a solid insoluble substrate or carrier having antibodies for a particular antigen bound to the carrier surface. A fluid thought to contain the antigen of interest is contacted with the carrier. If present, the antigen reacts with the carrier-bound antibody forming a complex. Free labeled antibody which also reacts with the antigen is added, simultaneously or subsequently, to the solution. The free labeled antibody reacts with the bound antigen. This two-part reaction results in an insoluble three-part complex in the form of a sandwich. The substrate-bound and the labeled antibodies form the "bread" while the

---

35 IMMU, p. 6.3.

36 FUND, p. 432-33.

37 FUND, p. 440.

38 FUND, p. 440.

39 FUND, p. 440.

40 FUND, pp. 433-440.

41 Engvall specification, p. 4.

antigen forms the "filler."[42] Where the label on the antibody is an enzyme, the assay falls into a category of immunoassays known as Enzyme-linked Immunosorbent Assays (ELISA).[43]

II. Written description

During the preliminary motion period, David filed a motion for judgment under 37 CFR § 1.633(a). Paper 32. The motion asserted, inter alia, that Engvall's claims 8 to 27 were unpatentable under 35 U.S.C. § 112, ¶ 1. Each of claims 8 to 27 include the limitation that the monoclonal antibodies have a specific minimum "affinity for the antigenic substance. . . ." David asserted that the minimum affinity constant limitation was not described in Engvall's specification. Paper 32, p. 8-18. An Administrative Patent Judge granted the motion, but deferred judgment until judgment could be awarded with respect to all claims corresponding to the count. Paper No. 77, p. 5-6. We find that Engvall's specification does not provide a written description for the lower limit of the affinity constant specified in claims 8 to 27.

A. Proceedings

1. Proceedings before the patent examiner

Engvall filed application 06/285,477 on July 21, 1981. The application included seven claims.[44] After rejection of all claims by the examiner, the application was abandoned in favor of continuation application 06/539,754. The continuation was filed on October 6, 1983. On March 7. 1984, Engvall filed a preliminary amendment in the continuation. Engvall Application 06/539,754, Paper 18. The amendment added claims 8 to 45. The amendment stated:

---

42 Engvall specification, pp 4-5; FUND, pp. 438-439.

43 FUND, pp. 438-440.

44 Claim 1, the only independent claim follows:

> A method for the determination of an antigen (I) in solution, in which determination said antigen (I) is reacted with antibody (II), which is directed against the antigen (I), and with an antibody (III), which is directed against the antigen (I) and is labeled with an analytically indicatable atom or group and is soluble in said solution in the presence of which the determination is carried out, to the formation of a conjugate comprising said antigen (I) and said antibodies (II) and antibody (III), which conjugate is insoluble or is made insoluble, whereafter the amount of said analytically indicatable atom or group precipitated form said solution is determined, wherein the improvement comprises in using as antibody (II) and antibody (III) in said determination antibodies which are monoclonal and react with sterically spaced determinants of the antigen (I).

> This amendment is made for the purpose of provoking an interference between the present application and David et al U.S. Patent No. 4,376,110.

Engvall Application 06/539,754, Paper 18, p. 8. Claims 8-27 were said to differ from claims 28-45 solely in the absence of the recitation of the "10^8 liters per mole limitation in the latter claims." Engvall Application 06/539,754, Paper 18, p. 8. The examiner rejected the claims including the affinity constant limitation, claims 8-27, under 35 U.S.C. § 112, ¶ 1. The examiner found that the specific minimum affinity constant limitation present in each claim was not supported by Engvall's written description. Engvall Application 06/539,794, Paper 19, p. 2. The examiner held that the remaining claims were unpatentable over the prior art. Engvall Application 06/539,754, Paper 19, p. 2-3. The remaining claims, all of which lacked the specific affinity limitation were rejected over prior art. Engvall Application 06/539,754, Paper 19, p. 4. With respect to the written description rejection the applicant submitted a declaration by Asta Bergland to show that the affinity constant for the antibodies used in Engvall's example 1 was at least 10^{10} liters per mole. The amendment stated:

> In conclusion, the declaration shows the Example 1 of the subject patent application discloses antibodies having an affinity constant which are higher than the minimum value of 10^8 liters per mole given in claim 1 of U.S. Patent No. 4,376,110 to David.

Engvall Application 06/539,754, Paper 22, p. 2. The examiner was apparently convinced because subsequently the application was forwarded to this board for declaration of an interference. Interference Initial Memorandum, Interference 101,769, Paper 1.

2. David's preliminary motion

This interference was declared on April 8, 1987, between Engvall and David and a third party, Gallati et al. During the preliminary motions period David filed a motion for judgment under 37 CFR § 1.633(a) asserting that Engvall's claims 8 to 27 were unpatentable to Engvall under 35 U.S.C. § 112, ¶ 1. David asserted that Engvall's specification did not include a written description of the specific minimum affinity constant limitation present in those claims. Paper 32, pp. 8-18. David also asserted that, as the copier of claims, Engvall had the burden of showing descriptive support for the copied subject matter by clear and convincing evidence. David's motion relied upon a then recently decided case, Martin v. Mayer, 823 F.2d 500, 505, 3 USPQ2d 1333, 1337 (Fed. Cir. 1987). Paper 32, pp. 9-10. David argued that Engvall's specification lacked literal support for the

affinity limitation. Paper No.32, pp. 8-10. In addition, David asserted that affinity limitation was not inherent in Engvall's specification. Paper 32, pp. 10-15. Engvall opposed David's motion, arguing (1) that the affinity limitation was not material (Paper 39, pp.3-8); and (2) that the limitation "at least 10^8 liters/mole" was inherent in the examples (Paper 39, pp. 12-20). Engvall stated "when a person skilled in the art repeats the examples, e.g. Example I, that person inevitably and necessarily obtains monoclonal antibodies having an affinity of at least about 10^8." Paper 39, p. 14.

3. The APJ's decision on David's preliminary motion

In granting David's motion the APJ stated:

> As pointed out by David, the burden falls on the copier of a limitation to establish the inherency of the limitation. The [APJ] agrees with David to the extent he argues that neither Engvall nor Gallati have sustained this burden to date.

Paper 77, pp. 5-6.

B. The burden of proof

Engvall's reply brief for final hearing asserts that the APJ's decision was clearly erroneous because the APJ incorrectly imposed the burden of proving descriptive support for the affinity limitation on Engvall. Engvall Reply Brief., pp. 5-6. Engvall now challenges the placement of the burden relying on Kubota v. Shibuya, 999 F.2d 517, 522, 27 USPQ2d 1418, 1422 (Fed. Cir. 1993), and Behr v. Talbot, 27 USPQ2d 1401, 1405 (Bd. Pat. App. & Int. 1992). Kubota and Behr were decided long after the APJ's decision. These cases are relied upon for the proposition that under the "new" interference rules[45] the burden of proof is always on the moving party. This argument was raised for the first time in Engvall's Reply brief. We ordinarily do not consider such arguments. Suh v. Hoefle, 23 USPQ2d 1321, 1323-24 (Bd. Pat. App. & Int. 1991). Cf. Kaufman Co. v. Lantech, Inc., 807 F.2d 970, 973 n.*, 1 USPQ2d 1202, 1204 n.* (Fed. Cir. 1986) (courts normally do not consider arguments made for the first time in reply briefs). However, because of unusual circumstances of this case we will address the issue.

---

[45] This interference was declared under the "new" rules promulgated December 12, 1984, and which took effect, February 11, 1985. 49 Fed. Reg. 48416 (December 12, 1984) reprinted at 1050 Official Gazette 385 (January 29, 1985).

David's motion asserted that, as the copier of claims, Engvall had the burden of showing descriptive support for the copied subject matter by clear and convincing evidence. David's motion relied on Martin, Paper 32, pp. 9-10. Martin expressly holds:

> It is . . . the copier of the claims . . . who has the burden of proving, by clear and convincing evidence, "the disclosure on which he relies supports the copied claims which became the interference counts." [Citations omitted.]

823 F.2d at 505, 3 USPQ2d at 1337. In granting David's motion the APJ agreed that the burden was on Engvall to establish descriptive support for the limitation. Paper 77, pp. 5-6.

The Martin interference was an "old" rule interference. This interference is conducted under the "new" rules. Section 1.638(a) of the new rules specifically require that oppositions to motions "include an argument why the relief requested in the motion should be denied." Engvall's opposition never challenged the assignment of the burden. Paper 39. Engvall's failure to raise the burden of proof issue in the opposition to David's motion effectively waived the matter. The APJ could not have made an error by failing to consider a matter which was not brought to his attention. See, Keebler Co. v. Murray Bakery Products, 866 F.2d 1386, 1388, 9 USPQ2d 1736, 1738 (Fed. Cir. 1989) (prescience is not a required characteristic of the board and the board need not divine all possible afterthoughts of counsel that might be asserted for the first time on appeal). However, in view of the apparent confusion as to the proper burden of proof during the proceedings in this interference, we have evaluated the issue de novo with the burden of proof on David as the moving party. Kubota, 999 F.2d at 522, 27 USPQ2d at 1422; Behr, 27 USPQ2d at 1405. Thus, in order for David to prevail on the motion, a preponderance of the evidence must show that the lower limit of the affinity limitation is not supported by Engvall's written description. In reaching our decision on this issue, we have reviewed the parties' motion papers (37 CFR § 1.655(a)), as well as the briefs on the issue and the parties' evidence relied upon.

C. Significance of the primary examiner's decision that the affinity limitation was inherent in Engvall example 1

Engvall asserts that the decision of the primary examiner that the affinity limitation is supported by Engvall's Example 1 is an interlocutory decision in this interference and under 37 CFR §1.655(a) is presumptively correct. Thus, Engvall argues that "the Primary Examiner's declaration

of this interference and the decision that claims 8-27 are patentable to Engvall is an interlocutory order which is presumed to be correct." Reply brief, p. 4 (emphasis original).

Engvall's assertion is simply wrong. Examiners do not declare interferences. Interferences are declared by APJ's. 37 CFR § 1.610(a). Thus, no interference exists until declared by an APJ, and, manifestly, there can be no interlocutory order until an interference is declared. Our view is consistent with 37 CFR § 1.601(q), which defines an interlocutory order as any action taken during the interference, which is not a final decision, by an APJ or the Board, including the declaration of the interference . Thus, any decision of the examiner holding Engvall's claims patentable is not an interlocutory order and does not have a presumption of correctness under 37 CFR §1.655(a).

In addition, decisions of a primary examiner during ex parte prosecution are likewise not binding on the Board of Patent Appeals and Interferences in inter partes proceedings. See Bloch v. Sze, 458 F.2d 137, 173 USPQ 498 (CCPA 1972) (Prior ex parte determination by the Board of Appeals that the specification had written descriptive support for the claims was not binding on the Board of Patent Interferences on the issue of right to make the claims). See also, Okada v. Hitotsumachi, 16 USPQ2d 178, 1790-91 (Com'r Pat. 1990).

D. Finding on written description

After reviewing the evidence, we find that the lower limit for the affinity constant appearing in Engvall's claims 8 to 27 is not described in Engvall's specification. Accordingly, we hold that Engvall's claims 8 to 27 are unpatentable under 35 U.S.C. § 112, ¶ 1.

E. Analysis

We have reviewed the motion papers (37 CFR § 1.655(a)) and hold that David's motion satisfied the burden. David's § 1.633(a) motion (Paper # 32, pp. 8-18) presents facts and reasoning sufficient to make out a prima facie case of lack of a written description. David correctly pointed out that express support for the affinity limitation was lacking in Engvall's specification. Paper #32, pp. 8-10. A fact Engvall does not dispute. In addition, David's motion addressed in detail why the affinity limitation was not inherent in Engvall's specification. David's motion, therefore, provided sufficient basis to find that Engvall's involved application, prima facie, did not meet the description requirement for the claimed subject matter. Our review of the record indicates that Engvall has failed to rebut the prima facie case. Our reasons follow.

1. Claim interpretation

For the purpose of the description requirement issue, Engvall's claim 17 is representative. We have reproduced claim 17 below, adding indentation for clarity:

> 17. In an immunometric assay to determine the presence or concentration of an antigenic substance in a sample of a fluid comprising
>
> forming a ternary complex of
>
> a first labeled antibody,
>
> said antigenic substance, and
>
> a second antibody, said second antibody being bound to a solid carrier insoluble in said fluid
>
> wherein the presence of the antigenic substance in the samples is determined by measuring either the amount of labeled antibody bound to the solid carrier or the amount of unreacted labeled antibody,
>
> the improvement comprising employing monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole for each of said labeled antibody and said antibody bound to a solid carrier. [Emphasis added.]

As we did with the count, we construe the phrase "at least about 10^8 liters/mole" as indicating a lower limit for the range of the affinity necessary to use the claimed invention. We interpret "10^8" to mean 1×10^8. The phrase "at least" indicates the stated affinity constant is a minimum value. The word "about" adds some imprecision and extends the minimum affinity constant to a value somewhat below 1×10^8. In other words, we interpret "at least about 10^8 liters/mole" to indicate a range beginning somewhat below 1×10^8 liters/mole and extending to 4.

a. The materiality of the affinity limitation

In opposing David's motion, Engvall argued that the affinity limitation of claims 8 to 27 is not material. Paper 39, pp. 3-8. Implicitly, Engvall asserts that the purported lack of materiality excuses Engvall from the written description requirements of 35 U.S.C. § 112, ¶ 1. The statute and the case law interpreting the statute are clearly to the contrary. The first paragraph of § 112 expressly requires that the "specification shall contain a written description of the invention...." It is well settled that the "invention is, for the purpose of the 'written description' inquiry, whatever is now claimed." Vas-

Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1564, 19 USPQ2d 111, 1117 (Fed. Cir. 1991). Thus, the materiality of the limitation is simply irrelevant to the written description requirement.

In this regard, we note that Engvall chose to file an amendment copying claims from the David patent in order to provoke this interference. However, there is no need to copy claims exactly to provoke an interference. An applicant only needs to have patentable claims in the application which are (1) clearly supported by the specification (37 CFR § 1.75(d)(1)) and (2) are directed to the "same patentable invention" as claimed by the patentee (37 CFR §§ 1.601(i) & (n) (1986)). An interference can exist even where the scope of the claims of the parties do not overlap. See Aelony v. Arni, 547 F.2d 566, 570, 192 USPQ 486, 489-90 (CCPA 1977) (a method for purifying a compound using cyclopentadiene held to be the same patentable invention as a method using butadiene, isoprene, dimethylbutadiene, piperylene, anthracene, perylene, furan and sorbic acid).

2. Precedent

The general test for determining whether later claimed subject matter is supported by an earlier written description is whether the disclosure of the application "reasonably conveys to a person skilled in the art that the inventor had possession of the claimed subject matter at the time of the earlier filing date." Eiselstein v. Frank, 52 F.3d 1035, 1039, 34 USPQ2d 1467, 1470 (Fed. Cir. 1995); Ralston Purina Co. v. Far-Mar-Co., Inc, 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985); In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983). The specification must provide information that clearly allows persons having ordinary skill in the art to recognize that the applicant invented the later claimed subject matter. Vas-Cath, 935 F.2d at 1563-64, 19 USPQ2d at 1116; In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). In Vas-Cath the court noted that the disclosure must "convey with reasonable clarity to those skilled in the art that, as of the filing date sought, [the applicant] was in possession of the invention." Vas-Cath, 935 F.2d at 1563-64, 19 USPQ2d at 1117. (Emphasis original.) The court went on to state that the "invention is, for the purpose of the 'written description' inquiry, whatever is now claimed." Vas-Cath, 935 F.2d at 1564, 19 USPQ2d at 1117. (Emphasis original).

The Federal Circuit has analogized the determination of whether there is written descriptive support in a specification to following a trail through the forest by looking for "blaze marks" on individual trees:

> Many years ago our predecessor court graphically articulated this standard by analogizing a genus and its constituent species to a forest and its trees. As the court explained:
>
> > It is an old custom in the woods to mark trails by making blaze marks on the trees. It is no help in finding a trail . . . to be confronted simply by a large number of unmarked trees. Appellants are pointing to trees. We are looking for blaze marks which single out particular trees.

Fujikawa v. Wattanasin, 93 F.3d 1559, 1570, 39 USPQ2d 1895, 1905 (Fed. Cir. 1996), quoting In re Ruschig, 379 F.2d 990, 994-95, 154 USPQ 118, 122 (CCPA 1967). "Precisely how close the original description must come to comply with the description requirement of Section 112 must be determined on a case-by-case basis." Eiselstein, 52 F.3d at 1039, 34 USPQ2d at 1470, quoting Vas-Cath, 935 F.2d at 1561, 19 USPQ2d at 1116, quoting In re Smith , 481 F.2d 910, 914, 178 USPQ 620, 623-24 (CCPA 1973).

The determination that newly added subject matter meets § 112's written description generally involves at least one of three factors. The first involves the situation where claimed language is literally stated in the specification, i.e., literal antecedence in the specification for the newly added subject matter. The description requirement is ordinarily met by a specification which describes the invention in the same words as the claims.[46] In re Bowen, 492 F.2d 859, 864, 181 USPQ 48, 52 (CCPA 1974). See also, Smith, 481 F.2d 910, 914, 178 USPQ 620, 623 (CCPA 1973); Snitzer v. Etzel, 465 F.2d 899, 902, 175 USPQ 108, 110-11 (CCPA 1972), appeal after remand, 531 F.2d 1062, 189 USPQ 415 (CCPA 1976); Martin v. Johnson, 454 F.2d 746, 751-52, 172 USPQ 391, 395 (CCPA 1972).

If the new limitation is not literally set forth , then it must next be determined whether the limitation was actually described although in different language. In re Wilder, 736 F.2d 1516, 1520, 222 USPQ 369, 372 (Fed. Cir. 1984) ("It is not necessary that the claimed subject matter be described identically . . . ."); In re Lukach, 442 F.2d 967, 968-69, 169 USPQ 795, 796 (CCPA 1971).

---

[46] We use the word "ordinarily" because under some circumstances identical antecedent language in the specification may not provide an adequate written description of the subject matter of the invention. Thus, with respect to claims covering DNA molecules, it has been held that there is not an adequate written description unless the structure of the DNA is disclosed. Regents of the Uni. of Cal. v. Eli Lilly and Co., 119 F.3d 1559, 1566, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997).

(The written description requirement does not require in haec verba antecedence in the originally filed application). However, where different language is relied upon for support, "the specification must contain an equivalent description of the claimed subject matter." Lockwood v. American Airlines, Inc., 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997); Wagoner v. Barger, 463 F.2d 1377, 1380, 175 USPQ 85, 86 (CCPA 1972).

Last, if neither explicit language nor equivalent language is present, then it must be determined if the newly claimed feature is inherently present in the specification. Therma-Tru Corp. v. Peachtree Doors Inc., 44 F.3d 988, 993, 33 USPQ2d 1274, 1276 (Fed. Cir. 1995) ("[T]he later explicit description of an inherent property does not deprive the product of the benefit of the filing date of the earlier application."). Proof of inherency requires evidence that the "necessary and only reasonable construction to be given the disclosure by one skilled in the art is one which will lend clear support to . . . [this] positive limitation. . . ." Kennecott Corp. v. Kyocera International Inc., 835 F.2d 1419, 1423, 5 USPQ2d 1194, 1198 (Fed. Cir. 1987) quoting Langer v. Kaufman , 465 F.2d 915, 918, 175 USPQ 172, 174 (CCPA 1972) quoting Binstead v. Littmann , 242 F.2d 766, 770, 113 USPQ 279, 282 (CCPA 1957). In Kennecott, 835 F.2d at 1423, 5 USPQ2d at 1198, the court noted:

> The court has generally applied this standard of the "necessary and only reasonable construction" as a basis for determining whether an application could, on the basis of an inherent property, support a limitation in an interference count. [Citations omitted.]

As noted by the CCPA:

> Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. [Citations omitted.] If, however, the disclosure is sufficient to show that the natural result flowing from the operation as taught would result in the performance of the questioned function, it seems to be well settled that the disclosure should be regarded as sufficient.

In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981), quoting, Hansgirg v. Kemmer, 102 F.2d 212, 214, 40 USPQ 665, 667 (CCPA 1939). Thus, it is not sufficient that a person following the disclosure might obtain the result set forth; it must inevitably happen. (Our emphasis). Dreyfus v. Sternau, 357 F.2d 411, 415, 149 USPQ 63, 66 (CCPA 1966); Crome v. Morrogh, 239 F.2d 390, 392, 112 USPQ 49, 50 (CCPA 1956).

Regardless of which factor is involved, it is important to keep in mind that subject matter that would have been obvious to one of ordinary skill in the art from the specification, or subject matter that one having ordinary skill in the art could ascertain without undue experimentation is not necessarily described. The Federal Circuit has recently stated:

> Thus, an applicant complies with the written description requirement "by describing the invention, with all its claimed limitations, not that which makes it obvious," and by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that set forth the claimed invention."

Regents of the Univ. of Cal. v Eli Lilly & Co., 119 F.3d 1559, 1566, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997) quoting Lockwood, 107 F.3d at 1572, 41 USPQ2d at 1966. As further noted by the Federal Circuit in Lockwood, 107 F.3d at 1571-72, 41 USPQ2d at 1966:

> Entitlement to a filing date does not extend to subject matter which is not disclosed, but would be obvious over what is expressly disclosed. It extends only to that which is disclosed. While the meaning of terms, phrases, or diagrams in a disclosure is to be explained or interpreted from the vantage point of one skilled in the art, all the limitations must appear in the specification. The question is not whether a claimed invention is an obvious variant of that which is disclosed in the specification. Rather, a prior application itself must describe an invention, and do so in sufficient detail that one skilled in the art can clearly conclude that the inventor invented the claimed invention as of the filing date sought.

See also, In re Blaser, 556 F.2d 534, 538, 194 USPQ 122, 125 (CCPA 1977) ("However, the flaw in this argument is that enablement and obviousness are not the issues; description of the invention is."); In re Winkhaus, 527 F.2d 637, 640, 188 USPQ 129, 131 (CCPA 1975) ("That a person skilled in the art might realize from reading the disclosure that such a step is possible is not a sufficient indication to that person that the step is part of appellants' invention"); Ruschig, 379 F.2d at 995, 154 USPQ at 123 ("While we have no doubt a person so motivated would be enabled by the specification to make it, this is beside the point for the question is not whether he would be so enabled but whether the specification discloses the compound to him, specifically, as something appellants actually invented.").

Engvall urges that the affinity for the monoclonal antibodies and alphafeto protein described in Example 1 of her specification are above 10^8 and therefore provide the written description required by § 112, ¶1, for the affinity limitation. Engvall states (Brief, p. 94):

> A single example in an application that explicitly or inherently meets every limitation of a claim is sufficient to support it. Limitations need not be expressly set forth in haec verba. Binstead v. Littman, 242 F.2d 766, 113 USPQ 279, 282 (CCPA 1975); Kennecott Corp. v. Kyocera International, Inc., 835 F.2d 1419, 5 USPQ2d 1194, 1198 (Fed. Cir 1987), cert. denied, 486 U.S. 1008, 100 L.Ed. 2d 198, 108 S.Ct. 1735 (1988).

While the second quoted sentence correctly states the law, Engvall cites no authority for the first sentence with respect to the satisfying the written description requirement. While a reduction to practice of a single embodiment within the scope of a generic count may be sufficient for the purpose of priority in an interference, a patent applicant must have support for the full scope of the claimed subject matter to meet the description requirement of 35 U.S.C. § 112, ¶ 1. Conservolite Inc. v. Widmayer, 21 F.3d 1098, 1100, 30 USPQ2d 1626, 1628 (Fed. Cir. 1994) citing Squires v. Corbett, 560 F.2d 424, 435, 194 USPQ 513, 520 (CCPA 1977). As noted by the CCPA in Squires:

> We conclude that for an applicant to have a right to copy a patent claim he must have support for the full scope of the claim. This conclusion rests on the recognition that the right to make a claim in a pending application, even for purposes of interference, depends, as it does with all pending claims, on compliance with the requirements of 35 USC 112, first paragraph. There is no other standard.

Thus, Engvall's specification, as filed, must provide information which would lead the person having ordinary skill in the art to the lower limit of the affinity constant of "at least about 10^8 liters/mole" to satisfy 35 U.S.C. § 112, ¶ 1.

3. Engvall's original specification and the lower limit for the affinity constant of "about 10^8 liters/mole"

There is nothing in Engvall's original specification which provides express language or any blaze marks indicating a preference or appreciation for any particular value of the affinity constant. Nothing in the specification conveys that Engvall viewed an affinity of "at least about 10^8 liters/mole" or any other magnitude of the affinity constant as being of any significance at all with respect to the claimed invention. While the specification mentions and uses the word affinity, a review of the

specification indicates Engvall never conveyed any concern with the magnitude of the affinity of the monoclonals and antigens used.

On the other hand, Engvall did have concern for the specificity of the monoclonals. Indeed, the reason stated in the specification for employing monoclonals in her assays was the high specificity of the these antibodies. Engvall Application 06/539,754, specification, p. 4, lines 13 to 25.

Engvall's specification begins by identifying that the technological field of the invention is sandwich assays involving the use of two antibodies which are active against the same antigen:

> [T]he present invention relates to a method for the determination of an antigen (I) in solution, in which determination said antigen (I) is reacted with an antibody (II), which is directed against the antigen (I) and with an antibody (III), which is directed against the antigen (I) and is labeled with an analytically indicatable atom or group and is soluble in the liquid in the presence of which the determination is carried out, to the formation of a conjugate comprising said antigen (I) and said antibodies (II) and (III), which conjugate is insoluble or is made insoluble, whereafter the analytically indicatable atom or group is determined in the insoluble or insolubilized conjugate and/or in the solution.

Engvall Application 06/539,754, specification, p. 1, lines 3-14. The specification goes on to indicate that assay methods using biospecific affinity reactions are well known. At page 2, lines 1-11, of her specification Engvall states:

> A great number of assay methods involving biospecific affinity reactions [are] known in which methods a first immunochemical reactant is reacted with a second immunochemical reactant exhibiting biospecific affin[ity] to said first reactant and then a third immunochemical reactant, which exhibits biospecific affinity to the first or the . . . second reactant (i.e., is an immunochemical counterpart to the first or the second reactant) is reacted with its counterpart to the formation of a conjugate comprising said first, second and third reactants, one of said reactants being labeled with an analytically indicatable atom or group of atoms.

Engvall further notes that in order to determine the presence of the second reactant, the labeled conjugate must be separated from the labeled but unreacted component. She indicates that this is commonly done by using, as one of the reactants, a component bound to an insoluble polymer. The labeled conjugate is removed by simply removing the polymer with the bound conjugate from

the solution. Engvall Application 06/539,754, specification, p. 2, lines 16-27. Published literature and patents are referred to as describing specific techniques of separation. Engvall Application 06/539,754, specification, p. 2, line 27 to p. 3, line 18. Labeling of antibody and antigen is stated to be well known and techniques of labeling are said to be generally known. Engvall Application 06/539,754, specification, p. 3, lines 19-28. According to the specification "assay methods involving biospecific affinity reactions may be grouped into two types of methods, viz, competitive methods and 'sandwich' methods." Engvall Application 06/539,754, specification, p. 4, lines 1-3.

Sandwich assays are described as involving a first reactant bound to a carrier. According to Engvall's specification, the bound reactant is placed into a sample containing a second reactant to be determined. The bound reactant complexes with the second reactant. The second reactant is then reacted with a labeled immunochemical counterpart forming a sandwich. Engvall Application 06/539,754, specification, p. 4, lines 8-12. Engvall notes some benefits and problems of sandwich assays with reference to the importance of specificity of the antibodies used:

> When determining an antigen in a sample a sandwich type method is often preferred since such a method does not require the use of labeled antigen which in certain cases is difficult to procure. However, the sandwich assay places particularly stringent requirements on the specificity of the antibody used and an assay procedure with multiple incubation and washing steps is usually required. [Emphasis added.]

Engvall Application 06/539,754, specification, p. 4, lines 13-19.

Engvall describes her invention as an improvement over these conventional sandwich assay methods. According to the express statements in the specification, the improvement resides in the use of monoclonal antibodies which react with sterically spaced determinants on the antigen. Thus, the specification states:

> According to the present invention an improved sandwich assay method of the type set forth in the introductory part above is provided, which method is characterized in using as antibody (II) and antibody (III) in said determination antibodies which are monoclonal and react with sterically spaced determinants of the antigen (I). [Emphasis added.]

Engvall Application 06/539,754, specification, p. 4, lines 13-19. In identifying the improvement over conventional sandwich assays affinity is not mentioned. The use of monoclonals is said to provide

benefits over prior art use of polyclonal antibodies. The prior art "polyclonal" antibodies are said to comprise "a population of different antibodies having varying specificities and affinities which antibodies can be directed against different sites [or] determinants on the antigen (I)." Engvall Application 06/539,754, specification, p. 5, lines 7-11. The use of monoclonals is said to allow more accurate analysis and analytical procedures which have not been possible before. Engvall Application 06/539,754, specification, p. 5, lines 11-15. In the preferred embodiment, monoclonal antibodies (II) and (III) react with structurally different determinants on the antigen. The advantage of this embodiment is said to be that it permits the simultaneous addition of both monoclonal antibodies to the antigen-containing solution. This eliminates an incubation and washing which is necessary with prior art procedures using polyclonal antibodies. Engvall Application 06/539,754, specification, p. 5, lines 18-25. Simultaneous addition of the two monoclonal antibodies is the preferred technique for practicing the embodiment. Engvall Application 06/539,754, specification, p. 5, lines 25-28. In discussing this preferred embodiment, neither affinity nor the affinity constant of the monoclonal antibodies is not mentioned.

In another disclosed embodiment, the antibodies react with determinants which are equal or structurally the same. In this embodiment the antibodies are reacted with the antigen sequentially. The antigen first is reacted with the bound antibody and subsequently reacted with the unbound and labeled antibody. Engvall Application 06/539,754, specification, p. 6, lines 1-13. In describing this specific embodiment neither affinity nor the affinity constant is mentioned.

With respect to the monoclonal antibodies useful in the invention, the specification indicates that the preparation of monoclonals is described in the prior art. The referenced prior art includes the seminal work of Köhler and Milstein. Engvall Application 06/539,754, specification, p. 6, lines 14-21. The specification also describes a general method for preparation of monoclonal antibodies. Engvall Application 06/539,754, specification, p. 6, line 22 - p. 7, line 10. The monoclonals may be bound to a carrier and labeled according to prior art techniques. Engvall Application 06/539,754, specification, p. 7, line 11 - p. 8, line 29. In describing the preparation of monoclonal antibodies, the affinity constant is not mentioned.

The specification next presents three examples which are said to further illustrate the invention. Engvall Application 06/539,754, specification, p. 9, lines 5-7. Examples 1 and 2 relate

to the detection of the antigen human alphafeto protein (AFP). Example 3 relates to the detection of another antigen, human fibronectin (HFN). Engvall Application 06/539,754, specification, pp. 9-15. The examples include a detailed explanation of the preparation of the respective monoclonal antibodies for the antigens, bonding of the monoclonals to a substrate, labeling of the antibodies and determination of the antigens. The examples do not mention affinity or the affinity constant of the antibodies and antigens used.

The original claims of the application which are, of course, part of Engvall's written description, are directed to a method for the determination of antigen. The claims are presented in Jepson format. The improvement is said to be in using as the monoclonal antibodies which react with sterically spaced determinants of the antigen. Again no reference is made to affinity. Engvall Application 06/539,754, specification, pp. 16-17.

Thus, Engvall's specification unquestionably discloses the use of monoclonal antibodies in sandwich assays, and, while making some general references to affinity, is devoid of any indication, appreciation or guidance that any particular value for the affinity constant was of importance. Engvall's specification contains no express statement or implicit description that would lead the person of ordinary skill in the art to use monoclonal antibodies and antigens having any particular value of the affinity constant.

4. Inherency of the lower limit of "at least about 10^8 liters/mole" based on the data in Engvall's example 1

Engvall does not urge that there is express language or equivalent language in the specification which provides a basis for the specific affinity constant limitation. Rather, Engvall asserts that the limitation inherently finds basis in her Example 1. Engvall Brief, pp. 94-102, Engvall Reply Brief, pp. 2-10.[47] At the outset, we note that if any of Engvall's examples had expressly stated that both antibodies used had an affinity constant of 10^8 liters/mole, Engvall may have had written descriptive support for adding a claim utilizing 10^8 liters/mole as a lower limit. Absent, an express or implicit statement, the evidence must show that the person having ordinary skill in the art repeating

[47] In this regard, Engvall's briefs are somewhat confusing due to the apparent failure to distinguish between claims and counts. The subject matter of the count is not relevant to the description requirement issue.

any of those examples would be led to this lower limit. Fujikawa, 93 F.3d at 1571, 39 USPQ2d at 1905; Oelrich, 666 F.2d at 581, 212 USPQ at 328

Engvall relies on the testimony of Drs. Langone[48] and Bergland to establish that the limitation "at least 10^8 liters/mole" is inherent in Example 1 of the specification. Engvall Reply Brief, pp. 7 to 10. Bergland and Langone each calculated an affinity constant from the data in Engvall's example 1. Bergland calculated the value to be $3.7x10^9$ liters per mole. E128, p. 5.[49] Langone using a different method arrived at a value of 5.2×10^9 liters per mole. ER 3515-19, E131. Accepting these calculations at face value, we fail to see how they would lead the person of ordinary skill in the art to the lower limit of "about $1x10^8$ liters/mole." While Bergland's and Langone's values fall within the scope of the subgenus of the present claims, i.e., they are "at least about 10^8 liters per mole," a subgenus is not necessarily described by a genus encompassing the subgenus and an embodiment on which the subgenus reads. In re Smith, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972). Rather, the specification must provide descriptive support for the full scope of the claimed subject matter. Conservolite, 21 F.3d at 1100, 30 USPQ2d at 1628; Squires, 560 F.2d at 435, 194 USPQ at 52. Precisely how close the original description must come to comply with the description requirement of Section 112 must be determined on a case-by-case basis." Eiselstein, 93 F.3d at 1039, 34 USPQ2d at 1470. Bergland's and Langone's values are 37 and 52 times higher, respectively, than the lower limit specified in the claims. In our view, these values are simply too distant from the lower limit of about $1x10^8$ to act as a "blaze mark" to direct the person having ordinary skill in the art to "at least about 10^8 liters/mole." As noted by the Federal Circuit the search

---

[48] David moves to suppress Langone's testimony as untimely and because Engvall's counsel allegedly precluded David's counsel from adequately cross-examining Langone by instructing the witness not to answer certain questions. David et al. Motion to Suppress Evidence (Paper 331), pp. 8-12. David's motion does not establish that Langone's testimony was actually untimely or improper. All David has provided is conclusory statements. In addition, the refusal to answer certain questions by Langone goes to the weight of the testimony not to its admissibility. Land v. Regan, 342 F.2d 92, 101, 144 USPQ 661, 669 (CCPA 1965). The motion as to Langone's testimony is denied.

[49] The designation"E" followed by a number is a reference to Engvall's exhibits and the exhibit number. E.g., E128 refers to Engvall Exhibit 128. The designation "ER" followed by a number refers to the Engvall Record and the specific page number of the record. The designation "DX" followed by a number refers to David's exhibits. The reference "DR" followed by a number refers to the David Record and the specific page number. The reference to "DCX" refers to David Cross Exhibits and the exhibit number.

is "for blaze marks which single out particular trees." Fujikawa, 93 F.3d at 1570, 39 USPQ2d at 1905.

Additionally, we view Bergland's and Langone's calculations based on the data in Engvall's example 1 as too speculative to give a reasonably reliable value for the affinity constant of the antibodies and antigens used in that example. The testimony indicates that there are wide discrepancies in the mathematical determination of affinity constants. Langone testified that the affinity constant calculation could vary by a factor of 10, plus or minus, when different methods of evaluation were used (ER 3855-58 and ER 4311-13). Bergland testified that a difference of 10-15 times could result depending on the quality of the data used in making the determination of the affinity constant.[50] ER 1764. Drs. Engvall and Ruoslahti testified that different techniques of determining the affinity constant will give you different affinity constant values. ER 3246-48; ER 2478-79.

In addition, we find that the calculations of the affinity constant made by Bergland and Langone from Engvall's example 1 data may well be questionable. As discussed below, one having ordinary skill in the art would not necessarily determine the same values for the affinity constant from the data in Engvall's example 1 as determined by Bergland and Langone. We find that there is insufficient information in Engvall's example 1 for a person having ordinary skill in the art to make a reliable determination of the affinity constant for either of the antibodies.

Lastly, Langone's and Bergland's calculations, to the extent that they are reliable at all, only apply to the liquid-phase antibodies. As we show below, the evidence indicates that it is not possible to mathematically determine the affinity constant for an antibody bound to a substrate.

---

[50] "I accept by such a method when we know that we are drawing, I mean, in a Sca[t]chard plot, if you will not have the points along a straight line, you can get variation up to, as I said, 10 to 15 times difference." ER 1764, lines 11-15.

a. Engvall's estimates of the affinity constant for the labeled liquid-phase antibody of example 1

i. Bergland's Scatchard Plot determination of the affinity constant

In her affidavit submitted under 37 CFR § 1.672(b) (E128), Bergland testified that she calculated the affinity constant of the labeled antibody from the data in Example 1 using an inverse "Scatchard Plot" analysis. The affidavit indicates she followed the procedure described in an article by William H.C. Walker (E57). E128, p. 3.

According to the Walker article, a Scatchard Plot is a graphical technique in which data obtained from an immunometric assay is mathematically transformed and plotted on a graph. From the graph, certain information about the underlying antibody/antigen reaction can be determined. For example, the technique may be used to determine the affinity constant (K) for the reaction. E57, p.588. Walker further indicates that, the inverse Scatchard Plot is most conveniently used by plotting the values of Lw_2 as the x-axis values and the values of B*/Ab* as the y-axis values. E57, p.588. L is the concentration of antigen and $w_2 = 1\text{-}Ab^*/B^*$.[51] Ab* is defined by Walker as the assay result for a sample having a large excess of ligand. E57, p. 388. B* represents the assay value for sample corrected for background. To obtain B*, the background level is subtracted from the value obtained from each assayed sample. E57, p. 588-89 and Table 1. In Walker's examples, the background is determined from a "zero standard" --a sample which does not contain any of the antigen to be measured. E57, p. 589, Table 1. Proper values for Ab* and B* are important in obtaining an accurate analysis. As noted by Walker, "[a]ppropriate limits must be set for B*, zero in the absence of ligand, Ab* in the presence of a great excess in ligand." E57, p.588. Thus, to carry out a Scatchard Plot analysis requires the following: (1) antigen concentration for each sample, (2) the result of the immunometric assay for each sample (the counts per minute[52] or absorbance), (3) the background counts or absorbance, and (4) the result of the immunometric assay for a sample having a great excess of antigen.

---

[51] Walker exemplified the Scatchard plot by using data obtained from an immunoradiometric assay. E57, p. 589, Tables 1 and 2. The assay used a radioactive label rather than an enzyme label as used in Engvall's examples. Accordingly, Walker's examples refer to counts bound rather than absorbance.

[52] Walker refers to this as the "counts bound." E57, p. 589.

Engvall's example 1 does not explicitly identify the background absorbance or the absorbance for a sample having a great excess of ligand.

a) B* and the background absorbance level

In performing the Scatchard analysis, Bergland assumed the background absorbance value to be .01. At page 3 of her declaration (E128), Bergland states:

> 9. Referring to Exhibit E57 at page 589, I prepared my table (Exhibit E55[53]) in a similar fashion. Thus, referring to the third column of the table, which gives values for B^*, this represents bound absorbance minus background. I used a value of 0.01 for the background, as that is the value that is generally accepted.

While Bergland thought .01 was appropriate, Dr. Langone had a different view of the appropriate background level. Dr. Langone also estimated the affinity constant from Engvall's example 1 data but using another technique. ER 3511-20. Dr. Langone, however. was of the view that .1, not .01, was the background absorbance level. Dr. Langone stated (ER 3514-15):

> I have taken a conservative approach in the sense that I have stated that the background, appropriate background for this experiment is an absorbance value of 0.1. . . .
>
> Q. Doctor, before you go ahead and tell us what you did to come up with that number, let me ask you; you said you took a conservative approach, and used, as the background value, 0.1, is that correct, that's what you did?
>
> A. That's correct.
>
> Q. Why did you do that, and what makes it conservative?
>
> A. I did that to be conservative in the sense that the binding affinity calculated at the 12.5 dose range would be lower than if we chose a dose of alphafeto protein less that. If you look at the alphafeto protein concentration on page 12 [of the Engvall specification], given as 6.5 micrograms per ml., and look at the corresponding absorbance, the absorbance is 0.13. That, to me, is a value close to 0.1, and for that reason, I chose to use a higher value of 12.5 micrograms per ml. for these calculations.

---

[53] The David et al. Motion to Suppress Evidence (Paper 331) , pp. 14-15) objects to this Exhibit along with Exhibit E56 as misleading. This argument goes to the weight of the evidence, not to its admissibility. The motion is denied as to those exhibits.

Other evidence suggests a background absorbance value of .02. Engvall's Example 1 includes a second set of data immediately following the data used by Bergland in her calculations. Engvall Specification, p. 12. The second set represents an experiment identical to the first except the monoclonal bound to the substrate and the labeled monoclonal were the same. The results show an absorbance of .02 at all antigen concentrations. This result is expected because both the bound and labeled monoclonals were directed against the same determinant. When the labeled antibody was added to the mixture there was nothing with which to react since the determinant had already been utilized in reacting with the bound monoclonal. Because no reaction with the labeled monoclonal is expected, the reported absorbance level might be a reasonable indicator of the background absorbance. On cross examination, Bergland confirmed that the additional Example 1 data indicates a possible background level of .02 (ER 1782):

> Q. You assumed in this calculation at that the background was .01 in your declaration, is that correct?
> A. Yes.
> Q. You say that is the normal background, is that correct?
> A. Yes, in our system.
> Q. Yet the data presented immediately below the data which you have manipulated here in example 1 of the patent application shows a uniform background level of .02, doesn't it?
> A. That is right.[54]

Langone similarly noted that the absorbance from the second set of data was a reasonable background level. Langone, ER 3500.

While not necessary for our decision, we illustrate below the significance of even a small change in the background absorbance level on the affinity constant determined using the Walker technique. We have repeated Bergland's calculations using .02, rather than .01, for the background absorbance. Using this value, with all other values as used by Bergland, we have determined an affinity constant of 3.6×10^{10} liters per mole for the information in Engvall's Example I. This value is an order of magnitude higher than that calculated by Bergland and about 360 times greater than

---

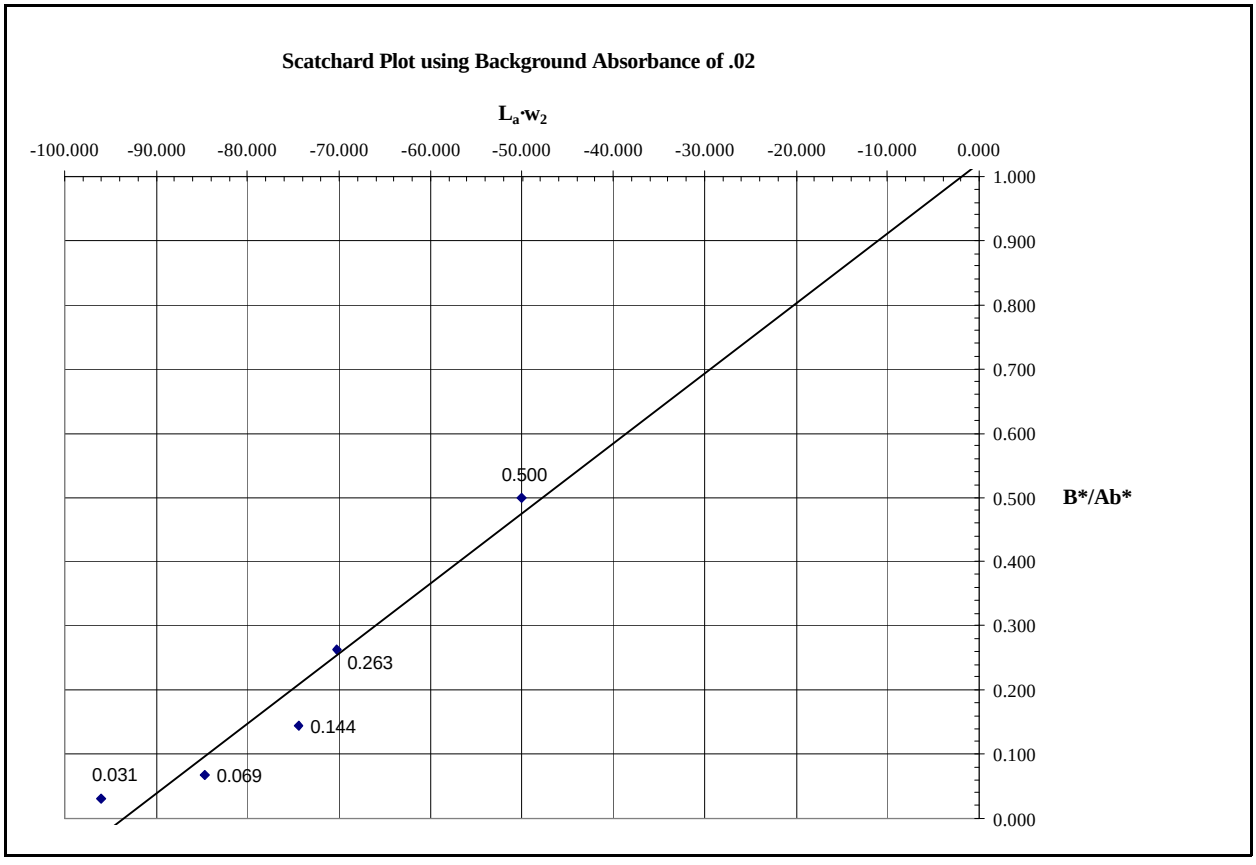
[54] Prior to answering the last question, the witness noted some confusion about the question. The following appears in the transcript immediately prior to the final answer (ER 1782):

> A. Can you repeat? I didn't understand.
> Mr. Lipsey: Why don't you read it back?
> (Record read.)

the lower limit set forth in the claim. The recalculated data and the Scatchard Plot are shown in the Table and in Graph 1 below:

Scatchard Plot Data from Example 1 using Background Absorbance of .02

L_a (µg/liter)	Absorbance	B*	B*/Ab*	Ab*/B*	w_2=1-Ab*/B*	$L_a \cdot w_2$
3.1	0.07	0.05	0.03125	32	-31	-96.100
6.25	0.13	0.11	0.06875	14.5455	-13.5455	-84.659
12.5	0.25	0.23	0.14375	6.9565	-5.9565	-74.457
25	0.44	0.42	0.2625	3.8095	-2.8095	-70.238
50	0.82	0.8	0.5	2	-1	-50.000
100	1.6					



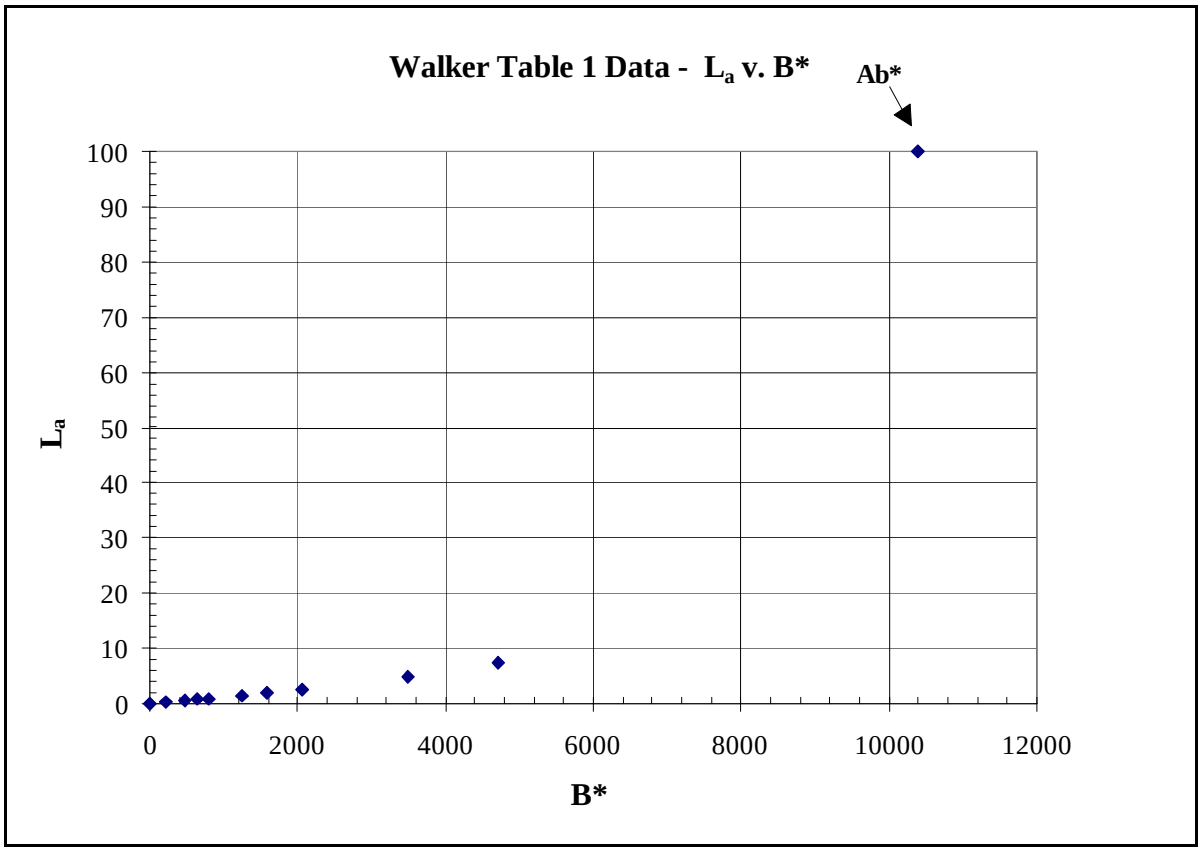
Graph 1

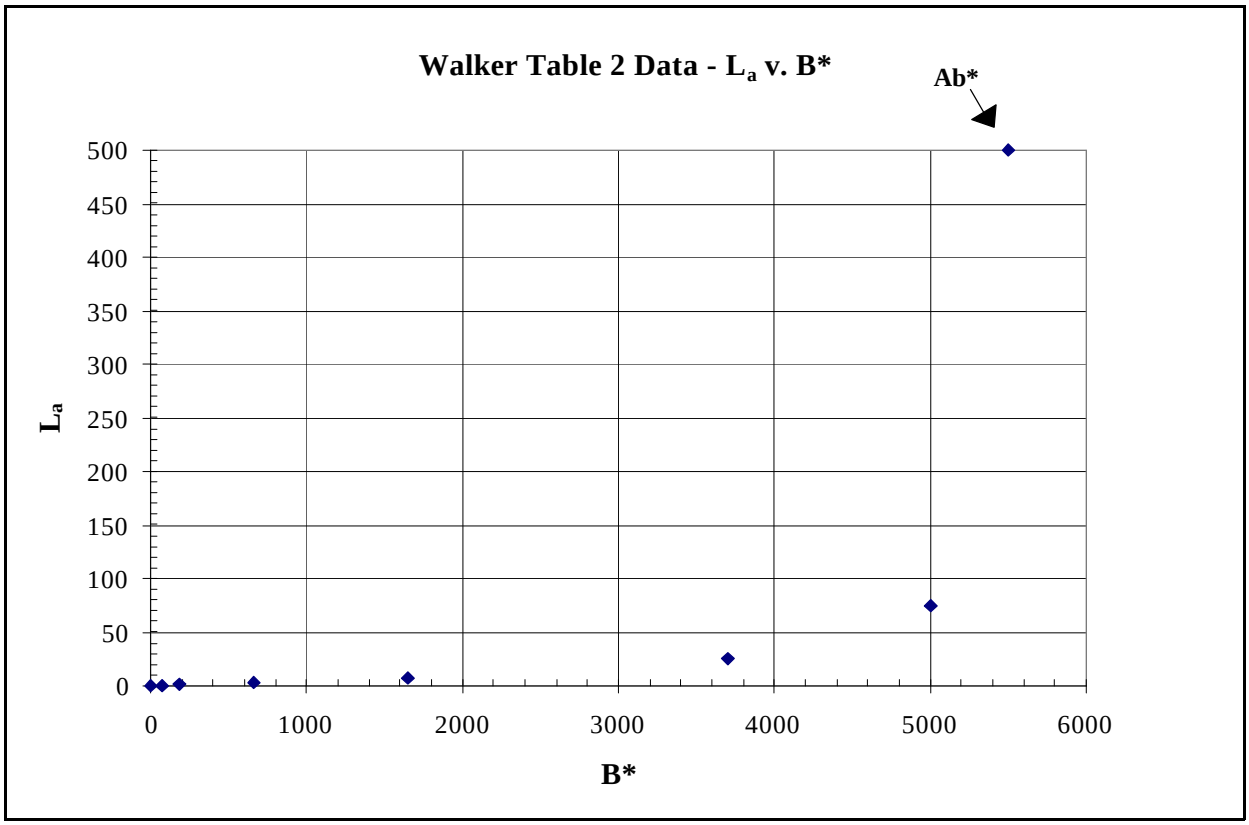
Based on the conflicting evidence before us, we are unable to determine what value the person of ordinary skill in the art would use as the background absorbance. Without knowledge of the appropriate background level, any determinations based on a Scatchard analysis, including the estimate of the affinity constant, are speculative.

b) The value of Ab*

Engvall's example 1 also fails to explicitly describe the value of Ab*, the assay result for a sample having a great excess of antigen. As we indicated above, the value of Ab* is significant because it is used in the calculations of both the x- and y-axis values that are used in the Scatchard Plot. Bergland used a value of 1.6 for Ab*, the maximum reported absorbance value reported in Engvall's example 1. E128, p. 3. We find however, that the person of ordinary skill in the art would not necessarily recognize 1.6 as an appropriate value for Ab*.

As noted in the Walker article, Ab* is the value for a sample with a great excess of ligand. E57, p 588. In other words, it is the immunometric assay result for a system which has nearly reached saturation --a condition in which a large increase in concentration (L_a) would have only a relatively minor effect on the assay value (B*). What Walker means by "a great excess of ligand" can be seen from Graphs 2 and 3 which we have prepared from the data in Walker's tables. E57, p. 589. Walker's Tables 1 and 2 are reproduced in the Appendix to this opinion.

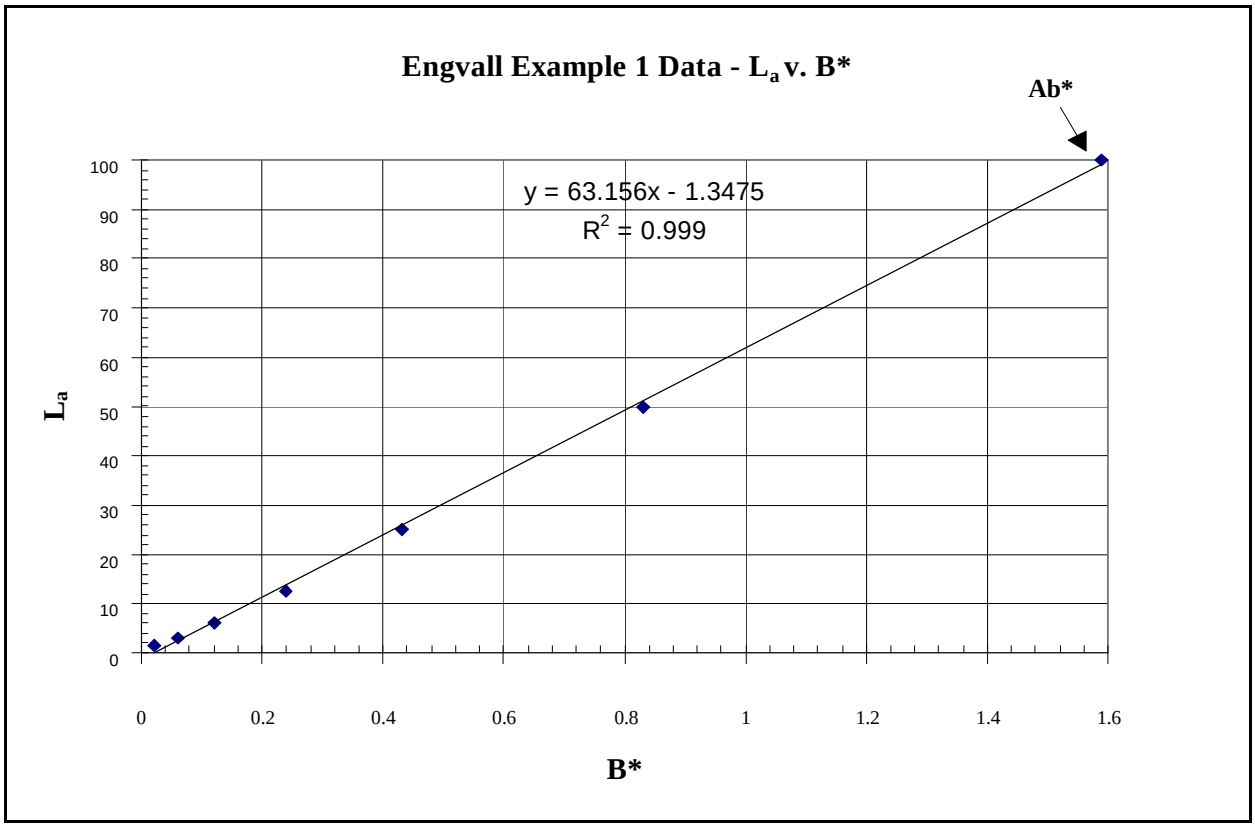

Walker Table 1 Data - La v. B*
Ab*
100
90
80
70
60
50
40
30
20
10
0
La
0
2000
4000
6000
8000
10000
12000
B*



Graph 3

The arrow in the upper right hand corner of each graph identifies Walker's Ab* data points, the points corresponding to a great excess of ligand. In Graphs 2 and 3, for the first nine and six data points, respectively, the increase in B^* is proportional to the increase in concentration L_a of the ligand, i.e., a relatively small increases in the concentration of antigen (L_a) results in a relatively large increases in the assay value B*. But for the Ab* data points shown in Graphs 2 and 3, a very large increase in concentration, L_a, results in a disproportionately small increase in the B* value. Thus, in Graph 3, a little over a 3-fold increase in concentration from 7.5 to 25 µg/liter resulted in a little over 2-fold increase in B*, from 1650 to 3700 counts per minute. The nearly 7-fold increase from 75 to 500 µg/liter, the Ab* concentration, resulted an increase of only ten percent (5000 to 5500 counts/minute).

For comparison we have also plotted Engvall's example 1 data on Graph 4 below.



Graph 4

The arrow at the upper right Graph 4 indicates the Ab* value of 1.6 used by Bergland, the highest value reported in Engvall's example 1. E128, p. 3-4. As can be seen from the plot, the Ab* value, 1.6, is proportional with the other reported values. In other words, the value of 1.6 is not near saturation and does not appear to be a assay value which represents a sample having a great excess of ligand. Engvall's value of 1.6 does not meet Walker's criteria of a limiting Ab* value. E57, p.588. Thus, the person having ordinary skill in the art would not know to a reasonable degree of certainty, what value to use for Ab*. The value for the affinity constant determined by Bergland from a Scatchard analysis of Engvall's example 1 data is speculative.

Thus, there is little certainty that the person having ordinary skill in the art would similarly arrive at a value of 3.7×10^9 liters/mole applying a Scatchard plot analysis to the data in Engvall's example 1.

ii. Langone's affinity constant calculation

Engvall also relies on the testimony of a Dr. Langone for the calculation of the affinity. Dr. Langone testified that he calculated the affinity constant from data available in example 1 of the Engvall specification. ER 3511-3520. Dr. Langone used a different method than Engvall to estimate the affinity. He used a technique based on information in chapter 6 of the Parker text book [55] (E130). Applying this technique to one of the data points in example 1 (12.5 µg/l), he calculated an affinity value for the labeled antibody of 5.2×10^9 liters per mole. ER 3519.

According to his testimony, Langone used the following relationship from the Parker text to calculate the affinity constant, (ER 3515):

$$S=1/K_a.$$

Langone testified that S in equation is sensitivity of the system and K_a is the approximate affinity constant. ER 3514-15. Langone identified the Parker text, particularly item 2 on page 137, as the source for this equation. ER 3515. Item 2 states:

> 2. Association (K_a) or avidity (K_{avid}) constants for antibody-hapten and antibody-protein interactions range between $1x10^4$ and $1x10^{13}$ liters/mole^{-1}. The practical sensitivity of an immunoassay is approximately equal to $1/K_a$ or $1/K_{avid}$. [Emphasis added.]

Parker further explains the equation and gives insight into the meaning of "practical sensitivity" (130, p. 111):

> As noted earlier, antibody affinity can vary over a broad range, resulting in marked variation in assay sensitivity. The importance of antibody affinity in hapten[56] binding can be illustrated by a simple calculation. In the law of mass action
>
> (1) $$K_a = \frac{(Ab \cdot H)}{(Ab)(H)}$$
>
> Under conditions in which 50% of the total hapten is antibody bound, Ab·H=H, and the equation reduces to

---

55 Charles W. Parker, Radioimmunoassay of Biologically Active Compounds, Chapter 6, "The Immunoassay, Thermodynamic and Kinetic Considerations," pp. 111-138, Prentice-Hall, Inc. 1976.

56 A hapten is a small functional group that corresponds to a single antigenic determinant of an antigen. FUND, p. 235.

(2) $$K_a = \frac{1}{(Ab)}$$

> In other words. The concentration of free antibody that must be maintained in order to bind 50% of the hapten is inversely proportional to K_a. In a competitive radioimmunoassay, it is not always necessary to achieve 50% binding of marker (although this amount is often what is used), but binding cannot be much less than 25% or the level of bound radioactivity will be too small. As an initial approximation, then, the K_a of the antibody determines the maximal dilution of antiserum than can be used to obtain adequate binding of the marker.

Thus, the practical sensitivity relates to a rule of thumb for determining maximal dilution which will give 50% antibody binding and insuring that there will be sufficient marker to bind to the antigen for detection.

Langone did not use Parker's equation to determine the practical sensitivity. Rather he used the equation in reverse to determine the affinity constant. From the data in Engvall's example 1, Langone selected a concentration of 12.5 µg/l and calculated a K_a of 5.2×10^9 liters per mole. ER 3515-19, E131. However, it is not apparent that a person having ordinary skill in the art would make the same selection in estimating the affinity constant. As indicated by the Parker text, the equation applies to the situation in which 50% of the antigen is bound to the antibody. E130, p.111. Langone did not establish or explain how he, or a person of ordinary skill in the art, would determine from the data in Engvall's specification that the value of 12.5 µg/ml in example 1 corresponds to 50% binding. Without this information, one having ordinary skill in the art would not have any basis to select 12.5 µg/ml or any of the other values in example 1, to give a reasonable estimate of the affinity constant.

In our view, the person of ordinary skill in the art would not necessarily arrive at a value of 5.2×10^9 liters/mole using Langone's technique. Thus, there is insufficient basis to find that the person of ordinary skill in the art would reach a reasonably similar determination of the affinity constant. In other words, Langone's determination is speculative and not the necessary and only reasonable construction to be given to the data in Engvall's example 1. Kennecott, 835 F.2d at 1423, 5 USPQ2d at 1198.

iii. Engvall's estimate of the affinity constant

Engvall further urges that because of the results of the assay shown in her example 1 measured nanomoles per liter (10^{-9} moles/liter) or less, the liquid phase antibody necessarily has an affinity greater than 10^9 liters per mole or higher. Engvall Brief p. 99-100; Reply Brief, p. 8. In this regard, Engvall testified (ER 3190):

> You know that if you can measure nanomoles per liter of an antigen, the affinity of your antibodies are going to be nanomoles per liter.

Even if Engvall is correct in this assertion, "10^9 or higher" does not reasonably and necessarily lead the person having ordinary skill in the art to use a lower limit of about 10^8 liters/mole.

b. The affinity constant of the carrier-bound antibody

With respect to the affinity constant for the antibody bound to a solid carrier, the evidence indicates that it is not possible to directly calculate the affinity constant from the data in Engvall's example 1. For example, the Scatchard Plot analysis only provides information about the labeled, liquid-phase antibody, not the carrier bound antibody. Thus, the Walker article indicates that the results of the Scatchard Plot analysis refer "to the labeled antibody in solution and are not related to the concentration or avidity of the antibody initially present on the solid phase." E57, p. 589. A publication by Rodbard et al. (GCX141[57]) indicates that the reaction system involved in an immunometric assay is extremely complex involving numerous rate constants and other factors. GCX141, p. 81, col. 1. Consistent with the Rodbard publication, Langone testified that "[t]he binding affinity for the immobilized antibody cannot be determined directly from the data given in example 1, because that antibody is immobilized on the plastic." ER 3495. Bergland similarly testified that it was not possible to determine the affinity constant for the antibody bound to a solid carrier. The following exchange took place during her cross-examination (ER 1980):

> Q. Turning now to your estimation of the affinity constant for the solid phase antibody in example 1 of the Engvall patent application, there is no mathematical way to derive the affinity constant, is there?
>
> A. No.

[57] "GCX" refers to Gallati Cross Exhibits. Gallati was a party to this interference. Gallati conceded priority and judgment was awarded against Gallati in Paper 281.

Apparently recognizing that the affinity of the carrier-bound antibody could not be calculated from the data in Engvall's example 1, Bergland and Langone expressed their opinions that the affinity of the constant for the carrier-bound antibody would be about the same as the enzyme labeled (liquid-phase) antibody. ER 1959; ER 3496. As we indicated above, the asserted value for the enzyme-labeled antibody was too speculative to provide reasonable direction to the person of ordinary skill in the art to constitute a written description of the lower limit of 10^8 liters per mole. Thus, their testimony that the affinity constant for the antibody bound to a solid carrier is about the same as the affinity constant for the liquid-phase antibody is equally speculative.

In any event, Bergland's and Langone's opinions as to the magnitude of the affinity constant of the carrier bound antibody are questionable. The Rodbard publication, in noting the complexity of the reaction system involved in with immunometric assays, cautions that "numerical evaluation of the curves is essential, and intuitive predictions are likely to be misleading." GCX141, p. 81, col. 1. In addition, the "affinity" of the antibody bound to a carrier may be artificially increased. Thus, David testified that in an ELISA type of assay

> antigen is applied, for example, to the bottom of a microtiter plate to create a solid-phase reagent which reacts with the monoclonal antibody. The microenvironmental concentration of antigen on the solid phase is artificially increased, causing the antigen to react with antibodies of lower affinity.

DR116[58]. David's testimony is consistent with other objective evidence. A publication titled " Antibodies A Laboratory Manual" (DCX83[59]) states the following:

> Antigens immobilized on solid supports at high concentrations promote high avidity, bivalent bonding.
>
> When an antibody binds to an antigen on a solid phase, the interaction is biphasic, and two factors, in addition to the intrinsic

---

[58] Engvall has moved for suppression of Dr. Gary David's declaration on the basis that is argumentative and misleading. Engvall et al. Motion under 37 C.F.R. § 1.656(h) to Suppress Certain Evidence Offered by David et al. (Paper 325), pp. 15-18 The motion is denied. The matters raised go to the weight of the evidence rather than its admissibility.

[59] Engvall has moved to suppress this document. Engvall et al. Motion under 37 C.F.R. § 1.656(h) to Suppress Certain Evidence Offered by David et al. (Paper 325), p. 12. The motion is denied. Engvall asserts that as a laboratory manual published in 1988 it is irrelevant and hearsay. We consider the document relevant to the opinions expressed by Langone and Bergland with respect to the affinity constant of the bound antibody. In addition, had we been asked we might have taken official notice of the scientific facts presented therein. FRE 201.

> affinity, control the strength of the interaction. These are high local concentration of the antigen and the possibility of bivalent binding. The initial binding of the antibody to the immobilized antigen is limited by diffusion, but after the first antibody-epitope interaction occurs, the formation of the second bond may be an intramolecular conversion if sterically possible (Fig. 3.5). In addition , the high local concentration of antigen increases the chance that any disassociated antibodies will rebind to neighboring antigens. In essence, diffusion occurs, but the high concentration of antigen acts as a trap to hold the antibody to the solid phase. These factors combine to yield a high avidity.
>
> This type of multimetric interaction can occur in cell staining, immunoblotting, and many types of immuno assays.

DCX-83, p. 33-34. The apparent increase in affinity for antibodies bound to a substrate is confirmed by Paul, Fundamentals of Immunology (FUND), p. 432-33.[60] This standard reference work states:

> However, another effect also increases the effective affinity in a two-phase system. This effect applies even for monovalent antibodies (Fab fragments) or monovalent ligands. The effect arises from the enormously high effective local concentration of binding sites at the surface compared to the concentration if the same number of sites were distributed in bulk solution. [Endnote omitted.]

Because of the possible enhanced affinity of the carrier-bound antibody, the person of ordinary skill in the art would not be able to reasonably conclude that the affinity of the bound antibody was necessarily about the same as that of the labeled antibody in solution from the overall sensitivity of the assay.

On pages 101 to 102 of her brief Engvall also argues to the effect that those working in the art would recognize that the antibodies used in Engvall's example 1 had affinities "higher than 10^8 liters/mole" (emphasis added) and refers to Langone's testimony to for support. However, Engvall's argument misses the mark. As we indicated above, the phrase "at least about 10^8 liters/mole" indicates the lower limit of the affinity constant for the invention. The evidence must show that one having ordinary skill in the art would be led to the lower claimed limit for the affinity constant of "at least about 10^8 liters/mole" from the examples not just to any affinity constant with some value higher than 10^8.

---

[60] See n. 5, supra.

In his testimony, Langone expressed the following opinions (Engvall Brief, p. 101-102): (1) that the hyperimmunization technique described in Example 1 for preparing the monoclonal antibodies bound to the substrate was a procedure that optimized the production of antibodies having an affinity of at least 10^8 liters/mole (Langone, ER 3503-11); (2) that the ELISA assay used to identify antibodies against AFP in example 1 removed low affinity antibodies resulting in antibodies having affinity constants "greater than 10^8 liters/mole" (Langone, ER 3527-28, 4236-38; Exhibit 130, page 137, ¶ 3); (3) the sensitivity of the assay and the fact that both antibodies had to have a similar affinity indicates that the example 1 antibodies have and affinity of "at least 10^8 liters/mole" (Langone, ER 3496-3502); and (4) that the relatively short time to complete the assay indicates affinities "greater than 10^8 liters/mole" (Langone, ER 3502-03, 3815-16; Exhibit E129, page 11, lines 26-29). We do not credit Langone's testimony since no objective evidence has been identified which indicates why the value for the affinity constant would be viewed as greater than 10^8 rather than greater than 10^6, 10^7, 10^9 or 10^{10} liters/mole. For example, Engvall has not pointed to any evidence that relates the time to complete the assay (Langone opinion (4)) to any quantitative value for the affinity constant. In other words, the evidence does not show that the person having ordinary skill in the art would not reach a conclusion that the affinity constant was "greater than 10^7," "greater than $10^{9,}$" or "greater than 10^{10}."

In any event, evidence of record contradicts most of Langone's opinions. With respect to hyperimmunization, Langone also testified that the hyperimmunization process does not always and necessarily make high affinity antibodies. Langone, ER 3873, line 6 - ER 3874, line 18. Thus, the record does not establish that a person having ordinary skill in the art repeating Engvall's example 1 would necessarily obtain high affinity antibodies for AFP using the hyperimmunization technique.

As to the ELISA assay removing low affinity antibodies, Engvall and her coinventors published an article (E67) discussing radioimmunoassay using monoclonal antibodies to AFP. The article was published in 1982, after Engvall's U.S. parent application filing date. Like Engvall's example 1, an ELISA assay was used to screen for anti-AFP activity. E67, p.11. Positive cultures identified by the ELISA assay were cloned and used for radioimmunoassay. E69, p.11. The authors report that this procedure did not always provide high affinity monoclonal antibodies. The article states:

> However, many of the monoclonal antibodies we have raised have had low affinities, giving assays of poor sensitivity.

E67, p. 16. Thus, one having ordinary skill in the art repeating Engvall's example 1, would not always and necessarily obtain high affinity antibodies.

Langone's third opinion, based on the sensitivity of the assay in Engvall's example 1 and that the affinities of both antibodies have to be about the same, is also contradicted. As we indicated above, Langone's calculation of the affinity based on the sensitivity of the assay is speculative. In addition the enhanced affinity effect of carrier-bound antibodies indicates that the affinity of both antibodies do not necessarily have to be the same to obtain a sensitive assay.

Accordingly, a preponderance of the evidence supports a finding that the minimum value for the affinity constant set out in Engvall's claims 8 to 27 is not inherent in Engvall's example 1. Engvall's claims 8 to 27 are not, therefore, supported by a written description and are unpatentable to Engvall under 35 U.S.C. § 112, ¶ 1.

III. Priority

A. The burden and standard of proof

As the junior party, Engvall bares the burden of proof on the issue of priority. Bosies v. Benedict, 27 F.3d 539, 541, 30 USPQ2d 1862, 1863 (Fed. Cir. 1994); Oka v. Youssefyeh, 849 F.2d 581, 584, 7 USPQ2d 1169, 1172 (Fed. Cir. 1988). "It is well settled that where an interference is between a patent that issued on an application that was copending with an interfering application, the applicable standard of proof is preponderance of the evidence." Bosies, 27 F.3d at 541-42, 30 USPQ2d at 1864, see also Peeler v. Miller, 535 F.2d 647, 651 n.5, 190 USPQ 117, 120 n.5 (CCPA 1976); Linkow v. Linkow, 517 F.2d 1370, 1373, 186 USPQ 223, 225 (CCPA 1975); Frilette v. Kimberlin, 412 F.2d 1390, 1391, 162 USPQ 148, 149 (CCPA 1969), cert. denied, 396 U.S. 1002 (1970). Where the junior party copies claims from a patent to provoke an interference the standard of proof is clear and convincing evidence where the patent issued before the junior party filed the application. Price v. Symsek, 988 F.2d 1187, 1190-91, 26 USPQ2d 103, 1036 (Fed. Cir. 1993).

David's patent issued on March 8, 1983. Engvall's involved application was filed on October 6, 1983. In a preliminary amendment to that application Engvall copied claims from David's patent to provoke an interference. Engvall Application 06/539,754, Paper 18. So Engvall's involved

application was not copending with David's application. However, the involved application is said to be a continuation of an earlier application filed on July 21, 1981. But when the interference was declared, the APJ did not give Engvall the benefit of the parent application. Paper 1. Engvall's preliminary motion for benefit was denied (Paper 77, p. 6) and Engvall has not requested review of that ruling at final hearing. 37 CFR § 1.655. Accordingly, the relevant standard in this case is clear and convincing evidence. However, we conclude that Engvall has not proved priority even by the less stringent standard of preponderance of the evidence.

B. Conception

1. Precedent

Conception is the formation "in the mind of the inventor of a definite and permanent idea of the complete and operative invention, as it is therefore to be applied in practice." Kridl v. McCormick, 105 F.3d 1446, 1449, 41 USPQ2d 1686, 1689 (Fed. Cir. 1997); Mahurkar v. C.R. Bard Inc., 79 F.3d 1572, 1577, 38 USPQ2d 1288, 1290-91 (Fed. Cir. 1996) Burroughs Wellcome Co. v. Barr Labs., Inc. , 40 F.3d 1223, 1228, 32 USPQ2d 1915, 1919 (Fed. Cir. 1994), cert. denied, 116 S. Ct. 771 (1996); Coleman v. Dines, 754 F.2d 353, 359, 224 USPQ 857, 862 (Fed. Cir. 1985); Gunter v. Stream, 573 F.2d 77, 80, 197 USPQ 482, 484 (CCPA 1978). The idea must be "so clearly defined in the inventor's mind that only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation." Mahurkar, 79 F.3d at 1597, 38 USPQ2d at 129; Burroughs; 40 F.3d at 1228, 32 USPQ2d at 1919 . A conception must include every feature or limitation of the count. Kridl, 105 F.3d at 1449, 41 USPQ2d at 1689. Thus, in order to establish conception, a party must show possession of every feature stated in the count, and that every limitation of the count must have been known to the inventor at the time of the alleged conception. Coleman, 754 F.2d at 359, 224 USPQ at 862; Davis v Reddy , 620 F.2d 885, 889, 205 USPQ 1065, 1069 (CCPA 1980). Each express limitation of the count is considered material and cannot be disregarded. Schur v. Muller, 372 F.2d 546, 551, 152 USPQ 605, 609 (CCPA 1967). In addition, "[i]t is well-settled that conception and reduction to practice cannot be established nunc pro tunc. There must be contemporaneous recognition and appreciation of the invention represented by the counts." (Emphasis original.) Estee Lauder Inc., v. L'Oreal, S.A., 129 F.3d 588, 593-94, 44

USPQ2d 1610, 1614 (Fed. Cir. 1997), quoting Breen v. Henshaw, 472 F.2d 1398, 1401, 176 USPQ 519, 521 (CCPA 1973).

2. Engvall's alleged conception

We conclude, that based on the record before us, Engvall has failed to prove a conception of the subject matter of the count.[61]

a. Engvall's alleged conception of the use of monoclonal antibodies having affinity constants of at least about 10^8 liters/mole

After careful review of the record and consideration of Engvall's arguments, we conclude that Engvall has not proved conception of every limitation of the count prior to August 4, 1980, the filing date of David's application. In particular, Engvall has not shown that she had possession of the conception of an immunometric assay where both monoclonal antibodies had affinity constants of "at least about 10^8 liters/mole" as specifically required by the count.

Engvall argues that the evidence shows the conception of the use of "high affinity" monoclonal antibodies. However, the weight of the evidence does not establish what the Engvall inventors considered to be a "high affinity." Implicit in Engvall's argument is that the generic phrase "high affinity" includes antibodies within the scope of the count. However, the fact that antibodies within the scope of the count arguably might be within the scope of the generic phrase "high affinity," that phrase does not constitute a definite description of monoclonal antibodies having an affinity constant of "at least about 10^8 liters/mole" as required by the count. See Bosies, 27 F.3d at 542, 30 USPQ2d at 1865 ("Although the compounds of the count arguably might be within the scope of the generic formula set out in Benedict's notebook, that formula does not constitute a definite description of the compounds of the count.").

---

[61] In the preliminary statement, Engvall asserts of a date of conception of mid-December 1978. We could interpret "mid-December" to mean December 16, 1978. This would be the earliest date upon which Engvall may rely in this proceeding. 37 CFR § 1.629(a) and (b). In her briefs, Engvall asserts a conception date of Fall of 1978. Fall, of course, runs from September 21 to December 21. However, Engvall is estopped from asserting any date earlier than set out in the preliminary statement. 37 CFR § 1.629(a) and (b); Dewey v. Lawton, 347 F.2d 629, 630-31, 146 USPQ 187, 188 (CCPA 1965). And where a time period is asserted, the date is presumed to be the last day of the period. Oka, 849 F.2d at 584, 7 USPQ2d at 1172. We could, therefore, use December 21 as the alleged date of conception. Additionally, Engvall apparently considers "Fall, 1978" to include dates prior to January 3, 1979. Engvall Brief, pp. 70-71. Thus, we could interpret Engvall's assertion of Fall, 1978, as an allegation of a date no earlier than January 3, 1979. However, regardless of which date is used, we hold that Engvall has failed to prove a conception of the subject matter of the count.

In her brief, Engvall summarizes the evidence of conception as follows (Engvall Brief, pp. 20-21):

> • Dr. Ruoslahti's 1978 Grant Applications described his plans to obtain high affinity antibodies to AFP, to have Uotila make monoclonal antibodies and test them, and to use monoclonal antibodies in diagnostic applications, including assaying for AFP.
> • In the Fall of 1978, Dr. Engvall was throughly familiar with the use of polyclonal antibodies in the sandwich assay, having taught it to undergraduate students, having published descriptions and illustrations of it, having carried out sandwich assays, having carried out sandwich assays herself, and having taught and supervised Susan Holbeck in carrying out sandwich assays.
> • Susan Holbeck, Esther Oh, and Dr. Edward Hayman each confirmed that in the Fall of 1978 Dr. Engvall came up with the idea of using such high affinity monoclonal antibodies in a sandwich assay. [Emphasis added, references to Engvall's brief deleted.]

Engvall's evidence is, in our view, insufficient to show that the inventors had an appreciation of the use of monoclonal antibodies having an affinity constant of at least about 10^8 liters/mole. Engvall argues at pages 14 to 19 of her reply brief that

> Engvall appreciated the need for "high affinity" monoclonal antibodies, that is, antibodies having an affinity constant of at least about 10^8 liters per mole.

In making this argument, Engvall notes that one of the inventors, Dr. Ruoslahti had been working with AFP for many years. Engvall also notes that concentration of 20 nanograms or more of AFP per milliliter was significant and any assay to be clinically useful would have to be sensitive enough to detect AFP in a concentration of 20 nanograms per liter. Counsel calculates this to be $3x10^{-10}$ moles per liter and using the rule of thumb from the Parker text, arrives at a minimum acceptable affinity constant of $3.3x10^9$ liters per mole for AFP. And since the determination of the affinity constant is allegedly only accurate to an order of magnitude, this translates to a constant of $3.3x10^8$ liters per mole. This value is within the scope of the count. Engvall also points to Dr. Uotila's testimony that she was looking for antibodies with a "high affinity" for AFP and that by "high affinity" she meant affinity which was comparable to the affinity of the best available conventional antiserum. Engvall reply brief, p 17. Engvall then states:

> The affinities of the polyclonals then in use in commercial AFP assays necessarily had to be sufficient to detect the minimum clinically significant concentration of AFP, namely, 20 nanograms of AFP per liter. That means that the affinities of those commercial polyclonals would be $3.3x10^9$ liters per mole or as low as $3.3x10^8$ liters per mole. An affinity of "at least about 10^8 liters per mole" is certainly "comparable to $3.3x10^8$ liters per mole.

Engvall reply brief, pp. 17-18.

The problem with this argument is that Engvall has not directed us to evidence of sufficient weight to prove by even a preponderance of the evidence that the inventors had a contemporaneous appreciation that 20 nanograms per liter was the clinically significant concentration of AFP or that detecting 20 nanogram per liter was a recognized goal of their research. Engvall has not identified any documentary evidence that shows this was an appreciated goal at the time of the alleged conception. The testimony of witnesses, speaking long after the fact from memory in regard to past transactions, in the absence of contemporaneous documentary or physical evidence, has been held to be of little probative value. Lockheed Aircraft Corp. v. United States, 553 F.2d 69, 75, 193 USPQ 449, 455 (Cl. Ct. 1977).

The reference to Holbeck's and Hayman's testimony (e.g. Engvall reply brief, p. 17, note 16) does not help Engvall's case. The referenced testimony, while apparently indicating that Holbeck and Hayman were aware that high affinity antibodies were desired, does not indicate Holbeck's and Hayman's understanding of the meaning of "high affinity" or that they associated the phrase "high affinity" with an ability to detect AFP in a concentration of 20 nanograms per liter or with an affinity constant for AFP of $3.3x10^8$ liters per mole. Thus, the statements of the Engvall inventors as to what they meant by "high affinity" antibodies are not corroborated.

The only testimony to which we have been directed that arguably correlates "high affinity" with "at least about 10^8 liters/mole" is that of John Langone. In particular, Engvall refers to Langone's testimony appearing at ER 3501-11. Engvall Brief, p.78. Langone there expresses his opinion as to the affinity constant of the antibodies used in example 1 from Engvall's specification. For example, Langone states:

> I feel confident in claiming that the binding affinity of both antibodies, including the antibody used to coat the plastic surface, in example 1

> is of relatively high affinity. That is with a binding affinity of at least 10 to the 8th.

ER 3505, lines 10-15.

We declined above (page 38) to credit Langone's testimony relating to his estimate of the affinity constants from the example 1 data. Other than the count of this interference, we have not been shown any objective basis for Langone's opinion that "relatively high affinity" means "at least 10^8 liters/mole."

More importantly, however, Langone's understanding of the affinity constant when he reviewed the data and testified in 1990, does not show that there was a contemporaneous recognition and appreciation of an affinity constant of "at least about 10^8 liters/mole" by the inventorsprior to August 4, 1980. Conception may not be shown nunc pro tunc. Estee Lauder, 129 F.3d at 593-94, 44 USPQ2d at 1614. Langone's 1990 testimony of his understanding of "relatively high affinity" fails to be probative of what was in the inventors' mind at the time of the alleged conception in the Fall of 1978. The question of conception is properly directed to whether there was "formation [] in the mind of the inventor of a definite and permanent idea of the complete and operative invention . . . [and whether] every limitation of the count [was] known to the inventor at the time of the alleged conception." Bosies, 27 F.3d at 543, 30 USPQ2d at 1865, quoting Coleman, 754 F.2d at 359, 224 USPQ at 862. Langone's understanding of the "relatively high affinity" is not probative of what was in the mind of the inventors. A junior party cannot satisfy the burden of proof and rebut the presumption in favor of the senior party on the basis of an incomplete written conception plus testimony of a non-inventor as to what the non-inventor thought the phrase "high affinity" meant. See, Bosies, 27 F.3d at 543, 30 USPQ2d at 1865. We have not been directed to any corroborating evidence which demonstrates the inventors' understanding of the meaning of "high affinity."

Engvall argues strenuously that the conception of the use of "high affinity" antibodies is sufficient to establish conception and that 10^8 liters/mole is not critical. However, "at least about 10^8 liters/mole" is an express limitation of the count. And all express limitations of the count are material and cannot be ignored. Schur, 372 F.2d at 551, 152 USPQ at 609. A party attempting to prove conception must prove conception of every express limitation of the count. Kridl, 105 F.3d at 1449,

41 USPQ2d at 1689; Coleman, 754 F.2d at 359, 224 USPQ at 862; Davis, 620 F.2d at 889, 205 USPQ at 1069; Schur, 372 F.2d at 551, 152 USPQ at 609.

Engvall relies on Vancil v. Arata, 202 USPQ 58, 60 (Bd. Pat. Int. 1977) to support the position that conception of "high affinity" is enough. In Vancil a panel of the former Board of Patent Interferences stated:

> Arata contends that Vancil has not established conception of the subject matter in issue because there is no evidence of conception of a collision sensor, which is one element recited in the counts. However, the law does not require that every element of the counts be conceived; rather, the test of conception is whether the disclosure by the inventor(s) was such that no extensive research or experimentation would be required for one of ordinary skill in the art to construct the invention in issue based upon that disclosure. Summers v. Vogel, 332 F.2d 810, 141 USPQ 816 (CCPA 1964); In re Tansel, 253 F.2d 241, 117 USPQ 188 (CCPA 1958); Mergenthaler v. Scudder, 11 App. D.C. 264, 276, 1897 C.D. 724, 731.

Vancil is not persuasive for two reasons. First, it does not appear to be binding precedent of this board. Second, and more importantly, it is inconsistent with the standard for proof of conception as set forth in binding precedent of the Federal Circuit and the former Court of Customs Patent Appeals. It is now well settled that conception requires proof of possession of every express limitation of the count. Kridl, 105 F.3d at 1449, 41 USPQ2d at 1689; Coleman, 754 F.2d at 359, 224 USPQ at 862; Davis, 620 F.2d at 889, 205 USPQ at 1069; Schur, 372 F.2d at 557, 152 USPQ at 609.

Engvall urges that to prove conception an inventor does not have to conceive the exact language of the count. Engvall relies on Silvestri v. Grant, 496 F.2d 593, 181 USPQ 706 (CCPA 1974) to support this argument and urges that the idea of the use of "high affinity" monoclonal antibodies is close enough for conception "of at least about 10^8 liters/mole." Engvall Brief, p. 77-78. In particular, Engvall relies on the following portion of Silvestri:

> This standard does not require that Silvestri establish that he recognized the invention in the same terms as those recited in the count. The invention is not the language of the count but the subject matter thereby defined. Silvestri must establish that he recognized and appreciated as a new form, a compound corresponding to the compound defined by the count. [Emphasis added.]

Silvestri, 496 F.2d at 599, 181 USPQ at 710.

In Silvestri, the board had held that Silvestri had not proved either conception or reduction to practice before the critical date. The count in Silvestri was directed to a specific chemical compound --a new form of ampicillin (Form II). The court stated:

> The ampicillin of the count is a new form of an otherwise old composition. It is now well settled that in such a case there is no conception or reduction to practice where there has been no recognition or appreciation of the existence of the new form.

Silvestri, 496 F.2d at 593, 181 USPQ at 708. After summarizing prior cases, the court set out the issue before it:

> The effect of these cases is that an inventor of a new form of an old composition cannot be accorded a date of invention earlier than the date when he recognizes the existence of the new form. Accordingly, the principal issue before us now is whether the evidence establishes beyond a reasonable doubt that, prior to Grant's filing date, Silvestri not only actually prepared Form II, but also appreciated that a new form of ampicillin had been obtained.

Silvestri, 496 F.2d at 597, 181 USPQ at 709. The court concluded that Silvestri proved conception and reduction to practice notwithstanding the fact that some of the express limitations of the count were not proved. The court stated:

> We believe that the results of the water assays and infrared analyses establish beyond a reasonable doubt that Silvestri had actually prepared a form of ampicillin corresponding to that obtained by Grant and thus to the count. In reaching this conclusion, we do not disregard the fact that the count also requires that the ampicillin possesses greater storage-stability than hydrated ampicillin and have a molecular weight of about 349. However, we regard these as inherent properties of Form II ampicillin which add nothing to the count definition beyond that determined by the water content and infrared spectrograph. In our view, these latter properties are sufficient to fully identify the new form of ampicillin. [Footnotes and citations omitted.]

Silvestri, 496 F.2d at 599, 181 USPQ at 709.

The Court concluded conception and reduction to practice existed because Silvestri demonstrated he (1) had actually made the specific ampicillin compound that was the subject matter of the count, and (2) contemporaneously recognized that the compound obtained was a new form of

ampicillin. It was not necessary, therefore, for Silvestri to prove express recognition of all of the "language" of the count because he proved he had actual possession of the specific compound that constituted the subject matter of the count and recognized the existence of that new composition. The court's conclusion was consistent with the long standing principle that a chemical compound and all of its properties are one and the same thing. In re Papesch, 315 F.2d 381, 391, 137 USPQ 43, 51 (CCPA 1963).

The facts here are substantially different than in Silvestri. Silvestri did not prove recognition and appreciation of two limitations of the count: (1) greater storage-stability than hydrated ampicillin and (2) a molecular weight of about 349. In concluding that Silvestri had actually reduced the invention to practice, the CCPA recognized this lack of proof but noted that the properties "add nothing to the count definition beyond that determined by the water content and infrared spectrograph." 496 F.2d at 599, 181 USPQ at 709. In other words, the references to storage-stability and molecular weight added information about the subject matter of the count, ampicillin, but did not change the scope of that subject matter. No subject matter was added or deleted from the count definition by the addition of the reference to storage-stability and molecular weight. With respect to the subject matter of this interference, the limitation "at least about 10^8 liters/mole" does not merely add further information. It effects the scope of the subject matter of the count. It eliminates from the subject matter of the count those monoclonal antibodies which have affinity constants for the antigen of interest of less than about 10^8 liters/mole. It is necessary, therefore, for Engvall to prove a contemporaneous recognition and appreciation that both monoclonal antibodies used in the alleged actual reductions to practice had an affinity constant of at least about 10^8 liters/mole.

We also note that Engvall, in arguing that a conception of the use of "high affinity" monoclonal antibodies is close enough, is, in effect, trying to amend the subject matter of the interference without filing an appropriate preliminary motion. Engvall is, in effect, trying to amend the count to read "employing monoclonal antibodies having a *high affinity* for the antigenic substance for each of said labeled antibody and said antibody bound to a solid carrier." The appropriate course of action for Engvall would have been to file a preliminary motion to amend the subject matter of the interference by substituting a count having the appropriate generic language. And Engvall had the

opportunity to move to redefine the subject matter of the interference. 37 CFR § 1.633(c). However, Engvall did not file a preliminary motion to substitute a count directed to the use generically of "high affinity" monoclonal antibodies rather than the specific minimum affinity specified in the current count. Engvall's burden in this interference is to show conception of each express element of the count as it currently exists. Coleman, 754 F.2d at 359, 224 USPQ at 862; Davis, 620 F.2d at 889, 205 USPQ at 1069.

We conclude that Engvall has not proved conception of the subject matter of the count prior to August 4, 1980. In our view, the evidence indicates, at best, only a general concern for "high affinity" antibodies. However, the subject matter of this interference requires a specified minimum for the affinity constant. Engvall has not proved by even a preponderance of the evidence conception of an embodiment of monoclonal antibodies having affinities of at least about 10^8 liters per mole.

C. Diligence

Engvall asserts diligence from prior to David's earliest alleged entry into the field, January 4, 1979, until an alleged reduction to practice in October, 1979. Engvall Brief, p. 68. Because we hold that Engvall has not proved conception, we do not address Engvall's alleged diligence.

D. Actual reduction to practice

Based on the record before us, we conclude that Engvall has not proved an actual reduction to practice of an embodiment within the scope of the count.

1. Precedent

An actual reduction to practice requires the existence of a physical embodiment within the scope of the count. Correge v. Murphy , 705 F.2d 1326, 1329, 217 USPQ 753, 755 (Fed. Cir. 1983); 1 C. Rivise & A. Caesar, Interference Law and Practice § 137 (1940). A party to an interference must show an appreciation or recognition by the inventor of the invention of the counts to establish a prior actual reduction to practice. In re Farrenkopf, 713 F.2d 714,720, 219 USPQ 1, 6 (Fed. Cir. 1983); Meitzner v. Corte, 537 F.2d 524, 190 USPQ 407 (CCPA 1976). The embodiment relied upon for an actual reduction to practice must include every limitation stated in the count. Schendel v. Curtis, 83 F.3d 1399,1402, 38 USPQ2d 1743, 1746 (Fed. Cir. 1996); Newkirk v. Lulejian, 825 F.2d 1581, 1582-83, 3 USPQ2d 1793, 1794 (Fed. Cir. 1987); Hummer v. Administrator of National Aeronautics & Space Administration, 500 F.2d 1383, 1387, 183 USPQ 45, 48 (CCPA 1974) (the

device must include every count limitation); Szekely v. Metcalf, 455 F.2d 1393, 1396, 173 USPQ 116, 119 (CCPA 1972) (all the limitations of the counts have to be satisfied). The evidence must also show that the embodiment is suitable for and actually worked for its intended purpose. Mahurkar, 79 F.3d at 1578, 38 USPQ2d at 1291; Scott v. Finney, 34 F.3d 1058, 1061, 32 USPQ2d 1115, 1118 (Fed. Cir. 1994); Newkirk, 825 F.2d at 1583, 3 USPQ2d at 1794; Wiesner v. Weigert, 666 F.2d 582, 588, 212 USPQ 721, 726 (CCPA 1981). In other words the embodiment must have a practical utility. Fujikawa, 93 F.3d at 1563, 39 USPQ2d at, 1898-99. Testing need not show utility beyond a possibility of failure, but only utility beyond a probability of failure. Scott, 34 F.3d at 1061062, 32 USPQ2d at 1118**;** Taylor v. Swingle , 136 F.2d 914, 917, 58 USPQ 468, 471 (CCPA 1943). And there is no requirement that the embodiment be in a "commercially satisfactory stage of development" to constitute a reduction to practice. Scott, 34 F.3d at 1063, 32 USPQ2d at 1118; DSL Dynamic Sciences Ltd. v. Union Switch & Signal Inc., 928 F.2d 1122 , 1126, 18 USPQ2d 1152, 1155 (Fed. Cir. 1991); King Instrument Corp. v. Otari Corp.767 F.2d 853, 861, 226 USPQ 402, 407 (Fed. Cir. 1985); Randolph v. Shoberg, 590 F.2d 923, 926, 200 USPQ 647, 649-50 (CCPA 1979).

In proving an actual reduction to practice, the inventor, must provide independent corroborating evidence in addition to his own statements and documents. Hahn v. Wong, 892 F.2d 1028, 1032, 13 USPQ2d 1313, 1317 (Fed. Cir. 1989). The corroboration "may consist of testimony of a witness, other than an inventor, to the actual reduction to practice or it may consist of evidence of surrounding facts and circumstances independent of information received from the inventor." Hahn, 892 F.2d at 1032-33, 13 USPQ2d at 1317; Reese v. Hurst, 661 F.2d 1222, 1225, 211 USPQ 936, 940 (CCPA 1981). When considering the sufficiency of corroborating evidence of an actual reduction to practice a reasonableness standard is used. Scott, 34 F.3d at 1061-62, 32 USPQ2d at 1118; Holmwood v. Sugavanam , 948 F.2d 1236, 1238, 20 USPQ2d 1712, 1714 (Fed. Cir. 1991).

2. Engvall's alleged actual reduction to practice[62]

Engvall alleges an actual reduction to practice by one of the inventors, Dr. Marjatta Uotila, prior to October 31, 1979. Dr. Uotila was hired to work in Dr. Ruoslahti's laboratory at the City of

---

[62] Engvall's preliminary statement alleges reduction to practice of on or about October 4, 1979. However, Engvall's brief alleges the date generally as "October, 1979." Since October encompasses a range of dates, it is appropriate to use the last day of the period, October, 31, 1979, as the alleged date of reduction to practice. Oka, 849 F.2d at 584, 7 USPQ2d at 1172. However, Engvall has failed to prove an actual reduction to practice.

Hope National Medical Center in June 1978. ER718, ¶ 8. During her tenure, in July of 1979, Dr. Ruoslahti moved his laboratory from the City of Hope to the La Jolla Cancer Research Foundation. Uotila, ER718, ¶ 10. She continued employment in Dr. Ruoslahti's laboratory until September, 1980. Uotila, ER718-19, ¶ 10. Dr. Uotila was assigned the task of developing monoclonal antibodies to AFP and to develop immunoassays using the antibodies. Uotila, ER718, ¶ 8. She states that she recorded her experimental work in six notebooks. Uotila, ER725, ¶ 22. She testified that she entered data into the notebook "generally" in chronological order. ER725, ¶ 22. She also indicated she only occasionally dated her work. Uotila, ER725, ¶ 22. Our review of the notebooks shows that the pages are rarely dated and that the notebook pages are neither signed nor witnessed.

It is alleged that Dr. Uotila carried out a one-step, two-site ELISA sandwich assay using two different monoclonal antibodies to human alphafeto protein. Engvall Brief, pp. 51-64. According to Engvall, Dr. Uotila performed several sandwich immunoassays for human alphafeto protein using two different monoclonal antibodies. Engvall Brief, pp. 51-62 and 80-94. The antibodies used were designated 50/3, 73/3 and 73/8. Engvall Brief, p. 59. Dr. Uotila testified that the raw data for the first successful sandwich assay is reported at page 0041 of her Notebook V (Exhibits 5 and 5A[63]) in the upper right hand corner under column 8. Uotila, ER759, ¶ 83; Uotila, ER760, ¶ 85. She said that she used this raw data to plot a dose response curve appearing in red in the center of page 0043D of Notebook V (Exhibit E5A). This data was replotted by Dr. Uotila during her redirect examination as Exhibit E109 and reproduced on page 58 of Engvall's Brief.

The portions of Engvall's brief directed to these alleged successful assays (Engvall Brief, pp. 51-68 and 80-94) do not mention the affinity constant of the antibodies used. Only a part of Engvall's Statement of Facts, Section II. F. (Engvall Brief, pp. 64-68), directly discusses the affinity constants of the antibodies used in the alleged reductions to practice. Engvall there relies only on subsequent testing to show that antibodies 50/3, 73/3 and 73/8 had affinity constants for AFP of at least about 10^8 liters per mole. These tests were performed ex parte in 1988, during the pendency of this interference, to show that antibodies 50/3, 73/3 and 73/8 met the "at about 10^8 liters per mole" limitation of the count. Engvall Brief, pp. 64-68. According to Engvall, samples of antibodies 50/3,

[63] E5 and E5A appear to be identical but the latter is a color rather than a black and white copy.

73/3 and 73/8 were allegedly stored from the time Dr. Uotila left La Jolla Cancer Research Institute until 1988. In June of 1988, these samples were sent to Asta Bergland to determine the affinity constants. Engvall Brief, p. 64. The affinity constants were reported to be $2.3x10^9$, $7.2x10^8$ and $6.5x10^8$ liters per mole, respectively. Engvall Brief p. 66-67. These values are within the scope of the count.

We have not been directed to any part of the record which shows that the affinity constants were actually determined contemporaneously with the one-step two-site sandwich assay. As we indicated above, an actual reduction practice requires that the embodiment include every limitation stated in the count. Schendel, 83 F.3d at 1402, 38 USPQ2d 1743 at 1746; Newkirk, 825 F.2d at 1582-83, 3 USPQ2d at 1794; Hummer, 500 F.2d at 1387, 183 USPQ at 48; Szekely, 455 F.2d at 1396, 173 USPQ at 119). The affinity constants of the antibodies used in the alleged reductions to practice were determined nearly ten years after the alleged successful assays.

David asserts that Bergland's determination of the affinity constants long after the alleged date of reduction to practice are "improper attempts to prove reduction to practice nunc pro tunc." (Emphasis original.) David Brief, p. 33.[64]

We agree with David that Engvall's reliance on the Bergland test results is an attempt to prove the affinity constants nunc pro tunc. As noted by the Federal Circuit in Estee Lauder:

> It is well-settled that conception and reduction to practice cannot be established nunc pro tunc. There must be contemporaneous recognition and appreciation of the invention represented by the counts. [Emphasis original.]

129 F.3d at 593-94, 44 USPQ2d at 1614, quoting Breen, 472 F.2d at 1401, 176 USPQ at 521 (emphasis added). Bergland's tests performed in 1988 do not demonstrate that there was

---

[64] David also moves to suppress the evidence of Bergland's alleged testing of the antibodies. David asserts that the evidence is inadmissible because it is nunc pro tunc. David et al. Motion to Suppress Evidence, pp. 3-4 (Paper 331). This motion is denied. Section 1.656(h) permits a party seeking a ruling on the admissibility of evidence to file a motion to suppress the evidence. Whether evidence constitutes an impermissible "nunc pro tunc" proof is not a matter of admissibility, but of weight. We have evaluated Engvall's nunc pro tunc evidence and have given it no weight.

David also asserts (David et a. Motion to Suppress Evidence, pp. 5-6) that Bergland's testimony on the testing of the antibodies should be suppressed because Engvall has not proved proof of a chain of custody for the tested antibodies and it has not been established that the antibodies Bergland tested are the same antibodies used by Dr. Uotila. The actual antibodies were not offered into evidence so it is unnecessary to prove a chain of custody. Whether the antibodies tested by Bergland were the same as used in the alleged reductions to practice goes to the weight of the evidence not to its admissibility.

contemporaneous recognition and appreciation that the affinity constants of antibodies 50/3, 73/3 and 73/8 were at least about 10^8 liters per mole at the time of the alleged reductions to practice in October, 1979, or prior to David's filing date of August 4, 1980. Engvall, therefore, has not proved an actual reduction to practice of an embodiment falling within the subject matter set out in the count prior to David's filing date.

Our discussion of Silvestri v. Grant at pages 45 to 47 of this opinion is relevant on this point also. As we noted there, the requirement of the count, that both monoclonal antibodies have an affinity constant of "at least about 10^8 liters/mole" is not merely superfluous extra information. It is a positive limitation which excludes subject matter from the scope of the count. It was necessary, therefor, for Engvall to prove recognition and appreciation of the affinity constant limitation.

In her reply brief, Engvall argues for the first time that Bergland's tests merely confirmed what the Engvall inventors knew all along. Engvall Reply Brief, pp. 28-29. Engvall asserts (Engvall Reply Brief, pp. 25-26):

> Engvall has demonstrated conception of the use of high affinity monoclonal antibodies in the Fall of 1978. Likewise, when Engvall's first successful sandwich assays were carried out in October 1979, Engvall recognized and appreciated that high affinity antibodies, i.e., having affinities of at least about 10^8 liters per mole, had been used.

More particularly, Engvall asserts that prior to the alleged actual reductions to practice in October, 1979, the inventors were looking for "high affinity" monoclonal antibodies comparable to the minimal clinically required affinity for conventional anti-AFP polyclonals; that Dr. Uotila had compared monoclonal antibodies including 50/3, 73/3, and 73/8 to conventional anti-AFP polyclonals and believed they were of high affinity comparable to "conventional" polyclonal antibodies; and that the inhibition assays run by Dr. Uotila told the inventors that antibodies 50/3, 73/3 and 73/8 had the "requisite affinity." In addition Dr. Engvall testified that in an assay that can detect antigen in amounts of nanomoles per liter the affinity constants for the antibodies are going to be nanomoles per liter. Engvall Reply Brief, pp. 26-28.

We view the above arguments, raised in Section II.B. of Engvall's reply brief (pages 25-29), to be new arguments. Such belated arguments do not give the opposing party adequate notice and a fair opportunity to respond. Section 1.656(b) of 37 CFR requires that all arguments be presented

in the junior party's principal brief. It is appropriate, therefore, for us to decline to consider these arguments. Suh, 23 USPQ2d at 1323-24.

Taking this view, we hold that Engvall has failed to prove that there was a contemporaneous appreciation and recognition of the affinity constants used in the alleged actual reductions to practice. Engvall, therefore, has not proved an actual reduction to practice of an embodiment meeting all the limitations of the count prior to August 4, 1980

In any event, our review of the matters raised in Engvall's reply brief indicates that we would not reach a different conclusion with respect to Engvall's alleged actual reduction to practice.

Engvall refers to pages 54 to 61 of her principal brief as setting out the facts and law demonstrating actual reductions to practice. Engvall Reply Brief, p. 51-64. However, that section of Engvall's brief does not address the affinity constant of the antibodies used in the alleged actual reductions.

With respect to the assertions that the Engvall inventors knew antibodies 50/3, 73/3, and 73/8 had "high affinity" comparable to clinically used polyclonals antibodies to AFP, (said to be at least $3.3x10^8$ liters per mole) (Engvall Reply Brief, p. 26), Engvall has not directed us to any evidence, which corroborates that "high affinity" meant comparable to $3.3x10^8$ liters per mole. Corroboration "may consist of testimony of a witness, other than an inventor, to the actual reduction to practice or it may consist of evidence of surrounding facts and circumstances independent of information received from the inventor." Hahn, 892 F.2d at 1032-33, 13 USPQ2d at 1317; Reese, 661 F.2d at 1225, 211 USPQ at 940. All of the evidence referred to relating to alleged contemporaneous knowledge of the affinity constants (pages 25 to 28 of the Reply Brief) are statements and documents of the inventors. There is no evidence independent of the inventor's information. Engvall elsewhere in the reply brief points to Holbeck's and Hayman's testimony, relied upon to corroborate conception of the use of "high affinity" antibodies (Engvall reply brief, p. 17, note 16). However, as discussed at page 43 of this opinion, their testimony does not indicate that they understood "high affinity" to mean at least $3.3x10^8$ liters per mole or an affinity constant comparable to clinically used polyclonal AFP antibodies. Their testimony indicates only that they were aware that "high affinity" monoclonal antibodies were desired. Their testimony did not relate "high affinity" to any particular affinity

constant value or relate it to clinically used polyclonal antibodies. Holbeck, ER 2124, lines 4-6; Hayman, ER 537, lines 2-9.

We conclude that Engvall has failed to prove an actual reduction to practice of an embodiment falling within the subject matter of the count, even if the improper arguments in Engvall's reply brief are considered.

IV. David's alleged inequitable conduct

Engvall contends that David's conduct during the prosecution which resulted in the issuance of the David patent and during this interference was inequitable and therefore precludes David from an award of priority. Engvall Brief, pp. 119-146. Engvall asserts two separate bases for inequitable conduct: (1) that David failed to disclose the "best mode contemplated by the inventor of carrying out his invention" and (2) David withheld material information from the PTO, namely, information that David did not assert criticality of the affinity constant limitation in the foreign counterparts of David's U.S. application.

We disagree with both of Engvall's assertions and hold that the record does not establish that David committed inequitable conduct during the proceedings before the PTO.[65]

A. Engvall's best mode theory

1. David's claimed subject matter

The David patent includes 29 claims all directed to a process for determining the presence or the concentration of an antigen. All of the claims require the use of two different monoclonal antibodies having an affinity constant of "at least about 10^8 liters/mole" for each. Representative claims 1, 10 and 19, the independent claims, are reproduced below:

> 1. A process for the determination of the presence or concentration of an antigenic substance in a fluid comprising the steps:
>
> (a) contacting a sample of the fluid with a measured amount of a soluble first monoclonal antibody to the antigenic substance in

[65] Entry of a judgment against an opponent based on inequitable does not entitle the party to a judgment on the issue of priority. See, e.g., Perkins v. Kwon, 886 F.2d 325, 12 USPQ2d 1308 (Fed. Cir. 1989) (one party not entitled to a patent because it lost on priority; the party winning on priority not entitled to a patent based on a prior public use/sale). Hence, the most Engvall could have achieved, had Engvall prevailed on the inequitable conduct issue, would be a judgment that David is not entitled to any claims. Engvall would not have prevailed on priority, because the claims are not patentable under 35 U.S.C. § 102(g)--whether the priority issue is raised by David inter partes in the interference, or by some other third party (including the PTO) in another proceeding.

order to form a soluble complex of the antibody and antigenic substance present in said sample, said first monoclonal antibody being labeled;

(b) contacting the soluble complex with a second monoclonal antibody to the antigenic substance, said second monoclonal antibody being bound to a solid carrier, said solid carrier being insoluble in said fluid, in order to form an insoluble complex of said first monoclonal antibody, said antigenic substance and said second monoclonal antibody bound to said solid carrier;

(c) separating said solid carrier from the fluid sample and unreacted labeled antibody;

(d) measuring either the amount of labeled antibody associated with the solid carrier or the amount of unreacted labeled antibody; and

(e) relating the amount of labeled antibody measured with the amount of labeled antibody measured for a control sample prepared in accordance with steps (a)-(d), said control sample being known to be free of said antigenic substance, to determine the presence of antigenic substance in said fluid sample, or relating the amount of labeled antibody measured with the amount of labeled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)-(d) to determine the concentration of antigenic substance in said fluid sample, the first and second monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole.

10. A process for the determination of the presence of an antigenic substance in a fluid comprising the steps:

(a) simultaneously contacting a sample of the fluid with first and second monoclonal antibodies to said antigenic substance, each monoclonal antibody having an affinity for the antigenic substance of at least about 10^8 liters/mole, said first monoclonal antibody being labeled and soluble in said fluid and being provided for in a measured amount and said second monoclonal antibody being bound to a solid carrier insoluble in said fluid, in order to form an insoluble complex of said first monoclonal antibody, said antigenic substance and said second antibody;

(b) separating said solid carrier from the fluid sample and unreacted labeled antibody;

(c) measuring either the amount of labeled antibody associated with the solid carrier or the amount of unreacted labeled antibody; and

(d) relating the amount of labeled antibody measured with the amount of labeled antibody measured for a control sample prepared in accordance with steps (a)-(c), said control sample being known to

> be free of said antigenic substance, to determine the presence of antigenic substance in said fluid sample, or relating the amount of labeled antibody measured with the amount of labeled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)-(d) to determine the concentration of antigenic substance in said fluid sample.
>
> 19. In an immunometric assay to determine the presence or concentration of an antigenic substance in a sample of a fluid comprising forming a ternary complex of a first labeled antibody, said antigenic substance, and a second antibody said second antibody being bound to a solid carrier insoluble in said fluid wherein the presence of the antigenic substance in the samples is determined by measuring either the amount of labeled antibody bound to the solid carrier or the amount of unreacted labeled antibody, the improvement comprising employing monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole for each of said labeled antibody and said antibody bound to a solid carrier. [Emphasis added.]

2. David's semi-automatic screening assay

Engvall asserts that the David inventors withheld the best mode of practicing their invention. In particular, Engvall alleges that in order to practice the claimed method, it is necessary to screen for monoclonal antibodies which have an affinity constant of at least about 10^8 liters per mole for the antigenic substance of interest . Engvall points out that David had a semi-automatic assay for performing this function when the David application was filed, that David acknowledged that the semi-automatic screening assay was the best approach to the screening procedure, and that David's specification did not disclose this semi-automatic technique. Engvall further alleges that David's disclosed a different less advantageous technique and that David desired to keep the semi-automatic assay technique a trade secret shows an intent to conceal the best mode. Engvall Brief, pp. 122-125.

3. Analysis

The first paragraph of 35 U.S.C. § 112 provides:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode

> contemplated by the inventor of carrying out his invention. [Emphasis added.]

The parameters of a best mode inquiry are set by the claims. Zygo Corp. v. Wyko Corp., 79 F.3d 1563, 1567, 38 USPQ2d 1281, 1284 (Fed. Cir. 1996); Engel Indus., Inc. v. Lockformer Co. , 946 F.2d 1528, 1531, 20 USPQ2d 1300, 1302 (Fed. Cir. 1991) ("The best mode inquiry is directed to what the applicant regards as his invention, which in turn is measured by the claims."); Chemcast Corp. v. Arco Indus. Corp., 913 F.2d 923, 927, 16 USPQ2d 1033, 1036 (Fed. Cir. 1990) ("The other objective limitation on the extent of the disclosure required to comply with the best mode requirement is, of course, the scope of the claimed invention."). Unclaimed subject matter is not subject to the disclosure requirements of § 112. Engel**,** 946 F.2d at 1531, 20 USPQ2d at 1302. See also Randomex, Inc. v. Scopus Corp., 849 F.2d 585, 588, 7 USPQ2d 1050, 1053 (Fed. Cir. 1988) ("It is concealment of the best mode of practicing the claimed invention that section 112 ¶ 1 is designed to prohibit") (emphasis in original).

David's claimed invention is a method for detecting and quantitating antigen in a sample using monoclonal antibodies which have a certain characteristic, the affinity constant for the antigen must be at least about 10^8 liters per mole. A screening assay is not a required step of David's method claims. David's semi-automatic screening assay is not necessary to practice the claimed invention. The antibodies identified by David's semi-automatic screening assay have not been shown to make the claimed method work any better. Indeed, Engvall has not directed us to any evidence which shows that the particular screening assay has any impact at all on the operation of David's claimed method. The fact that the semi-automated assay may give David an advantage over competitors is simply of no relevance to David's claimed invention. David's semi-automatic screening assay is simply not a mode or embodiment of the claimed invention and it was not necessary for the semi-automatic screening assay to be disclosed in David's specification.

Engvall argues that the disclosure of the best mode may require disclosure of features which are not claimed. Engvall relies (Engvall Brief, p. 122; Engvall Reply Brief, p. 55) on the following portion of Chemcast (913 F.2d at 928, 16 USPQ2d at 1037):

> A patent applicant must disclose the best mode of carrying out his claimed invention, not merely a mode of making and using what is claimed. A specification can be enabling yet fail to disclose an

> applicant's contemplated best mode. Indeed, most of the cases in which we have said that the best mode requirement was violated addressed situations where an inventor failed to disclose non-claimed elements that were nevertheless necessary to practice the best mode of carrying out the claimed invention. [Citations omitted.]

We do not disagree with Engvall that the best mode requirement may require the disclosure of non-claimed subject matter. However, for the reasons we have already stated, we do not believe this is such a case. The facts here are unlike those in Chemcast. In Chemcast, the claimed subject matter was a grommet for sealing openings in panels. The claims specified that the grommet have a locking portion and a base portion and specified certain characteristics about the materials for each. 913 F.2d at 924-25, 16 USPQ2d at 1034. The district court had held one of Chemcast's claim's invalid for failure to disclose the best mode. In particular the district court found that Chemcast had not disclosed "(1) the particular type, (2) the hardness, and (3) the supplier and trade name, of the material used to make the locking portion of the grommet." Chemcast, 913 F.2d at 926, 16 USPQ2d at 1035. The Federal Circuit affirmed because the inventor knew and had in mind a specific material for the locking portion of the grommet that was "necessary for satisfactory performance" of the invention. 913 F.2d at 928, 16 USPQ2d at 1037. In other words, Chemcast kept information to itself which it knew would effect how well the claimed invention worked. David's semi-automatic screening assay has not been shown to have any impact at all on the performance of the process set out in David's claims. David's screening assay has not been shown to result in antibodies which make the claimed process better or achieve a better result.

We do not view Spectra-Physics Inc. v. Coherent Inc., 827 F.2d 1524, 3 USPQ2d 1737 (Fed. Cir. 1987) as inconsistent with our view. In Spectra-Physics the patent claimes a laser. The laser comprised cups inside of a tube and

> means for attaching the distal edge of each of the cup rims along the inside wall of said tube.

827 F.2d at 1527 n.2, 3 USPQ2d at 1739 n.2. The preferred means for attaching each cup to the tube was a brazed joint between the edge of the cups and the tube. The court held that the patentee had violated the best mode requirement by failing to disclose the specific six stage brazing cycle which the inventor's used to join each cup to the tube. The court noted that the six-stage cycle produced

a reliable braze joint. 827 F.2d at 1531, 3 USPQ2d at 1741. In holding that the best mode was not disclosed the court also relied upon following legal conclusion of the district court:

> 3. The six stage braze cycle employed by Coherent, and developed by it, are [sic, is] necessary to the enjoyment of the invention taught by the patents in suit by a person skilled in the art of laser construction, and are [sic] not sufficiently disclosed by the patents in suit. [Bracketed material and second emphasis original. First emphasis added.]

827 F.2d at 1537, 3 USPQ2d at 1746. The six stage brazing cycle, while itself not required by the claims, directly relates to the quality of the brazed joint, i.e., the claimed "means for attaching the distal edge of each of the cup rims along the inside wall of said tube." In other words, the six stage brazing cycle had an impact on the quality of this particular element of the claimed subject matter.

David's semi-automatic assay for identifying antibodies is not necessary for the enjoyment of the David invention. David's semi-automatic assay as not been shown to impact the quality of the claimed assays. The antibodies identified by the semi-automatic assay have not been shown to give a better assay or have any impact at all on the process set out in David's claims.

We find that David did not violate the best mode requirement of 35 U.S.C. § 112, ¶ 1. In view of our holding that the best mode requirement was not violated, there can be no inequitable conduct for failure to comply with the best mode requirement.

B. Engvall's inconsistent positions theory

Engvall argues that both before the patent examiner and before this board, David asserted that the "at least about 10^8 liters/mole" limitation was "required," "material," and "critical," to patentability. Engvall Brief, p. 132. Engvall also asserts that notwithstanding these representations, David sought and procured patent claims in foreign countries which did not include any affinity limitation. In Engvall's view, these two positions are irreconcilable and inconsistent and, in failing to inform either the patent examiner or this board of the positions taken in foreign patent offices, David has violated the duty of disclosure to the PTO. Engvall Brief, p. 132.

In our view, taking different, irreconcilable and inconsistent positions before the PTO and foreign patent offices coupled with the failure to disclose the different positions to the PTO may support a conclusion that the duty of disclosure has been violated. David does not deny that broader claims were asserted and obtained in foreign jurisdictions. However, this fact standing alone does

not prove that David took irreconcilable and inconsistent positions. Whether or not the respective positions are irreconcilable and inconsistent depends on a variety of factual considerations. These include, but are not necessarily limited to: (1) the standards of patentability in the foreign jurisdictions, including applicable standards for "obviousness"; (2) the prerequisites for a reference to be considered prior art in the foreign jurisdictions; (3) the principles of claim construction in the foreign jurisdiction, including the impact of statements in the specification on the scope of the claim; (4) the prosecution history of the foreign applications including the rejections made, the references relied upon against patentability, and the arguments made in response to the rejections; (5) the procedures available in the foreign patent offices to overcome initial determinations of unpatentability; and (6) the similarity between the rejections made in the PTO and in the foreign patent offices. Without this factual background it is not possible to determine if inconsistent and irreconcilable positions were taken. For example, amendments and arguments made in a U.S. application to secure allowance of the claims may not be necessary where the principal reference relied upon in the U.S. is not prior art in the foreign jurisdiction. Thus, narrowing amendments, arguments, or evidence showing that a particular limitation was critical would not be necessary in the foreign jurisdiction. Under these circumstances, as well as many others, the fact that claims without a "critical limitation" were pursued and secured in the foreign jurisdiction is not inconsistent or irreconcilable with the position taken in the United States.

Here, Engvall has provided neither the necessary background information and evidence nor explained how the positions taken in the foreign patent offices and in the PTO are irreconcilable and inconsistent. We are unwilling to find that inconsistent positions were taken based solely on the fact that broad claims lacking a "critical" limitation were pursued and obtained in foreign jurisdictions.

We find that Engvall has not proved that David's assertion of broader claims in foreign patent offices is inconsistent and irreconcilable with the assertions of criticality of the affinity constant made in the proceedings in the PTO. Accordingly, we hold that on the record before us, David has not violated the duty of disclosure to the PTO.

FINAL JUDGMENT

Judgment as to the subject matter of the sole count in this interference is awarded against Junior Party Engvall et al. Eva S. Engvall, Erikki I. Ruoslahti and Marjatta Uotila are not entitled to a patent including claims 1 to 45 of their application 06/539,754 corresponding to the sole count of this interference. On this record, Gary S. David and Howard E. Greene are entitled to claims 1 to 29 of their U.S. Patent 4,376,110 corresponding to the sole count of this interference.

MARY F. DOWNEY Administrative Patent Judge	)	
FRED E. McKELVEY Senior Administrative Patent Judge	)	BOARD OF PATENT APPEALS AND INTERFERENCES
RICHARD E. SCHAFER Administrative Patent Judge	)	

cc: Maurice B. Stiefel, Esq.
Bryan, Cave, McPheeters & McRoberts
245 Park Avenue
New York, NY 10167-0034

Charles E. Lipsey, Esq.
Finnegan, Henderson, Farabow,
Garrett & Dunner
1300 I Street, N.W.
Washington, DC 20005-3315

APPENDIX

Tables 1 and 2 from Engvall Exhibit E57

Moore, Walter J., Physical Chemistry, 3d Ed., pp. 168-202, Prentice-Hall, Englewood Cliffs, New Jersey, 1964 (PHYCHEM)

Lewis, John R., First-Year College Chemistry, 7th Ed., pp.136-37, Barnes & Noble, New York, 1964 (CHEM)

Paul, William E., Fundamental Immunology, 3d Ed., pp. 422-433, Raven Press, New York, 1993 (FUND)

Roitt, Ivan et al., Immunology, 3d Ed., pp. 1.6-1.7 and 6.1-6.7, Mosby, London, 1993 (IMMU)

Watson, James et al., Recombinant DNA, 2d Ed. 1982, Scientific American Books, distributed by W.H.Freeman & Co., New York (DNA)

Darnell, James et al., Molecular Cell Biology, 2d Ed. 1990, Scientific American Books, distributed by W.H.Freeman & Co., New York (CELL)

Table 1. Data from Two-Site Ferritin Immunoradiometric Assay

L_a, µg/litre	Counts bound	B^*	B^*/Ab^*	w_2	$L_a w_2$	Calculated L_a, µg/litre
0	118	—	—	—	—	—
0.25	332	214	0.021	−47.6	−11.9	0.25
0.5	584	466	0.045	−21.3	−10.7	0.54
0.75	768	650	0.063	−15.0	−11.3	0.76
1.0	909	791	0.076	−12.1	−12.1	0.93
1.5	1360	1242	0.119	−7.4	−11.1	1.50
2.0	1700	1582	0.152	−5.6	−11.1	1.95
2.5	2175	2057	0.198	−4.0	−10.1	2.61
5.0	3610	3492	0.336	−2.0	−9.9	4.93
7.5	4838	4720	0.454	−1.2	−9.0	7.51
100	10 500	10 400	1.0	—	—	—

L_a = standard concentration in assay tube; B^* = counts bound to stationary phase − counts bound in zero standard; Ab^* = maximum counts bound; $w_2 = 1 - Ab^*/B^*$.

Table 2. Data from Two-Site Immunoradiometric Assay for Somatotropin

L_a, µg/litre	Counts bound	B^*	B^*/Ab^*	w_2	$L_a w_2$	Calculated L_a, µg/litre
0	250	—	—	—	—	—
0.25	318	68	0.013	−76	−19.0	0.25
0.75	433	183	0.033	−29	−21.9	0.65
2.5	910	660	0.119	−7.4	−18.5	2.4
7.5	1900	1650	0.30	−2.3	−17.5	6.8
25	3950	3700	0.68	−0.47	−11.8	23.8
75	5250	5000	0.91	−0.11	−8.3	78
500	5750	5500	1.0	—	—	—

Tables 1 and 2 from E57 (Walker, "The Scatchard Plot in Immunometric Assay," 23 Clinical Chemistry 588, 589 (1977))